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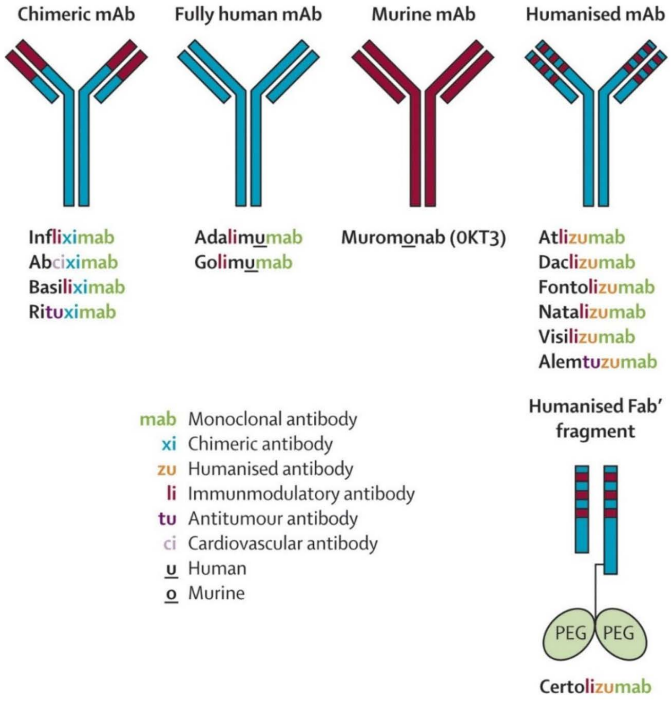

EFFECTS OF ANTI-TUMOUR NECROSIS FACTOR-α AGENTS ON POSTOPERATIVE OUTCOME IN PATIENTS WITH CROHN'S DISEASE UNDERGOING BOWEL RESECTION

BY
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AALBORG UNIVERSITY
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1. European Society of Coloproctology **collaborating group**. Patients with Crohn's disease have longer postoperative stay at hospital compared to patients with colon cancer undergoing abdominal resections. (in press)
2. Iesalnieks I., Spinelli A., Frasson M., Di Candido F., Scheef, B., Horesh N., Iborra M., Schlitt, H.J., **El-Hussuna A**., Risk of postoperative morbidity in patients undergoing bowel resections for colonic Crohn's disease. (accepted for publication in Techniques in Coloproctology.
3. Khazen B., **El-Hussuna A**, The use of perioperative supra-physiological dose of glucocorticoid is not supported by evidence: Systematic review. Dan Med J. 2018;65(6):A5488).
4. European Society of Coloproctology **collaborating group**. Risk factors associated with unfavourable post-operative outcome in patients with Crohn's disease undergoing abdominal resections. Colorectal Dis. 2017. (ahead of print)

5. Zangenberg M., **El-Hussuna A**. Psychiatric Morbidity after Surgery for Inflammatory Bowel Disease: A Systematic Review. World J Gastroentrol 2017. (ahead of print)
6. Zangenberg M., Horesh N, Kopylov U, **El-Hussuna A**. Pre-operative optimization of patients with Inflammatory Bowel Disease undergoing gastrointestinal surgery: Systematic review. Int J Colorectal dis 2017; 32: 1663-1676.
7. Hendel K, Kjærgaard S, **El-Hussuna A** A systematic review on pre, peri and post-operative factors and their implications for the length of resected bowel segments in patients with Crohn's disease. Int J Surg Open 2017; 7: 10-16.
8. European Society of Coloproctology **collaborating group**. The relationship between method of anastomosis and anastomotic failure after right hemicolectomy and ileo-caecal resection: an international snapshot audit. Colorectal Dis. 2017. (ahead of print)
9. **El-Hussuna A**., Hadi S, Iesalnieks I. No difference in postoperative outcome after acute surgery whether the patients presented for first time or are known with Crohn's disease. Int J Surg Open; 2017; 6: 1-4.
10. **El-Hussuna A.,** Iesalnieks I, Hadi S, Horesh N, Dreznik Y., Zmora O. The effect of pre-operative optimization on postoperative outcome in Crohn's disease resections. Int J ColoRectal Dis. 2017; 32:49-56.
11. **El-Hussuna A,** Lauritsen M & Bülow S. Complications following construction and closure of loop ileostomies - a systematic review. Ugeskr Laeger. 2011 May 30; 173(22):1563-1567.
12. **El-Hussuna A,** Lauritsen M & Bülow S. Relatively high incidence of complications after loop ileostomy reversal. Dan Med J. 2012; 59:A4517.

ENGLISH SUMMARY

Although the primary treatment for Crohn's disease (CD) is medical, more than half of patients with Crohn's disease will require surgery at some point during their lifetime. The risk of surgery at 1, 5, and 10 years after the diagnosis of CD is 16.3%, 33.3%, and 46.6%, respectively. The advent of anti-TNF-α agents has caused a therapeutic paradigm shift in the treatment of CD: the goal has changed from the management of disease flare-ups using immunosuppressive agents to the achievement of complete remission of the inflammatory state. However, the inhibition of TNF-α by anti-TNF-α agents might impair surgical wound healing and thus increase the rate of postoperative complications. There is therefore a need to investigate this risk in depth.

Aim

The aim of this thesis was to investigate the effect of treatment with anti-TNF-α agents on the postoperative outcome in patients with CD undergoing bowel resection.

Methods

Study 1 was a retrospective cohort multi-centre study using data collected from the hospital registry. The primary outcome was anastomosis-related complications or intra-abdominal septic complications (IASC) defined as overt anastomotic dehiscence, enteric fistula, or intra-abdominal abscess requiring treatment with laparotomy, laparoscopy or percutaneous radiologically guided drainage, and the secondary outcome variables were postoperative septic complications and other complications.

Study 2 constituted a systematic review and meta-analysis based on a predefined study protocol that was developed and reported according to the recommendations in the Cochrane Handbook for Reviews of Interventions and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Electronic searches of MEDLINE, EMBASE, and the Cochrane Library were performed. The primary outcome measure was anastomotic complications, including overt anastomotic leak (dehiscence), intra-abdominal abscess or enteric fistula verified by radiological imaging, laparotomy or laparoscopy. The secondary outcome measures were the total number of complications, non-anastomotic surgical complications, infectious complications, major medical complications, minor medical complications, reoperation and mortality.

Study 3 investigated the differences in reviews and meta-analyses regarding the effects of anti-TNF-α on postoperative outcome. This narrative systematic review included a literature search similar to that used in study 2 of this thesis. The primary outcome measure was 30-day postoperative complications.

Study 4 was a prospective, observational multi-centre cohort pilot study of the surgical stress response in patients with inflammatory bowel disease and investigated the effects of anti-TNF-α agents on the surgical stress response. The primary outcome

was the change in the concentration of immunological biomarkers of the surgical stress response (TNF-α, IL-6, and IL-10), and the secondary outcome measures were changes in IL-8, IL-17A, C-reactive protein, white blood cells, cortisol, transferrin, ferritin, and D-dimer, 30-day postoperative complications and length of postoperative stay in the hospital.

Results

The results of the first study showed that preoperative treatment with anti-TNF-α or immunomodulators does not affect the incidence of anastomotic complications, but other studies on the subject reached different conclusions. The study also confirmed previous findings that corticosteroids and colocolic anastomosis are associated with postoperative complications.

These findings stimulated an extensive search to find and pool all the available studies on the effect of anti-TNF-α agents on postoperative outcome. The subsequent meta-analysis of the identified studies concluded that in studies with a low risk of bias, anti-TNF-α agents increased the risk of anastomotic complications. Several meta-analyses on the topic have been published, and these yielded divergent results. Study 3 explained the reasons for the controversial findings of these meta-analyses. Meta-analyses with large numbers of patients and a quality assessment revealed that patients receiving anti-TNF-α treatment are at increased risk of overall postoperative complications.

Study 4 was designed based on the above-mentioned knowledge. This study showed no effect of anti-TNF-α treatment on the surgical stress response. However, the study also showed a wide variation in the type, dose, and duration of treatment, the drug concentration and the presence of anti-drug antibodies in patients who received anti-TNF-α agents during the 12 weeks prior to the operation. This variation might explain the conflicting results of retrospective studies regarding the effect of anti-TNF-α treatment.

Conclusion

The effect of anti-TNF-α therapy on the postoperative outcome in patients with CD results from an interplay of many factors. Some of the most important factors include concomitant steroid therapy, disease severity, nutritional status, smoking, preoperative optimization, preoperative drug concentration and the presence of anti-drug antibodies. Preoperative withdrawal of anti-TNF-α is not supported by the current evidence, but a multi-centre randomized controlled trial is needed to confirm or disprove this practice.

DANSK RESUME

Patienter med Crohns sygdom behandles som standard medicinsk, men mere end halvdelen af patienterne vil have brug for kirurgisk behandling en eller flere gange i løbet af deres levetid. Sandsynligheden for større abdominal kirurgi er henholdsvis 16.3%, 33.3% og 46.6% henholdsvis 1, 5, og 10 år efter diagnosen er stillet. Udviklingen af anti-TNF- α -lægemidler har medført et terapeutisk paradigmeskifte i behandlingen af Crohns sygdom. Målet har ændret sig fra håndtering af sygdommens opblussen med immunosuppressive lægemidler til opnåelse af fuld remission af den inflammatoriske tilstand. Studier har dog indikeret, at hæmning af TNF- α muligvis kan influere på kirurgisk sårheling og således øge risikoen for postoperative komplikationer. Der er derfor et behov for yderligere undersøgelser af denne risiko.

Formål
Formålet med denne afhandling er at undersøge effekten af anti-TNF-α lægemidler på postoperative outcomes hos patienter med Crohns sygdom, der gennemgår tarmresektion.

Metoder
Afhandlingen er baseret på fire studier:
Studie 1 er en retrospektiv multi-center undersøgelse. Data er indsamlet ved brug af hospitalsregistre. Det primære outcome er anastomose-relaterede komplikationer eller intra-abdominale septiske komplikationer (IASC), defineret som åbenlys anastomose lækage, enterisk fistel eller intra-abdominal absces, der kræver behandling med laparotomi, laparoskopi eller perkutan radiologisk vejledt drænage. De sekundære outcomes er postoperativ septisk komplikation og andre komplikationer.
Studie 2 er en systematisk gennemgang og meta-analyse baseret på en foruddefineret studieprotokol udviklet og rapporteret i overensstemmelse med anbefalingerne i Cochrane ”Handbook for reviews of Interventions” og ”Preferred Reporting Items for Systematic Reviews and Meta-Analyses” (PRISMA) erklæringen. De elektroniske søgninger blev udført i MEDLINE, EMBASE og Cochrane Library. Det primære outcome er anastomotiske komplikationer (AC), herunder anastomose lækage, intra-abdominale abscesser eller enteriske fistler, der blev verificeret gennem radiologisk billeddannelse, laparotomi eller laparoskopi. De sekundære outcomes er det samlede antal komplikationer, ikke-anastomose relaterede kirurgiske komplikationer, infektiøse komplikationer, større medicinske komplikationer, mindre medicinske komplikationer, re-operation og dødelighed.
Studie 3 undersøger forskellene i review og meta-analyser for effekten af anti-TNF-α lægemidler på postoperative outcomes. Undersøgelsen er en systematisk gennemgang uden statistisk analyse. Den litteratursøgning, der blev anvendt, var den samme som i studie 2. Det primære outcome er 30 dages postoperative komplikationer.
Studie 4 er et prospektivt, observationelt multi-center pilot studie. Det beskriver det kirurgiske stressrespons hos IBD-patienter og undersøger effekten af anti TNF-α

lægemidler på kirurgisk stressrespons. Det primære outcome er ændringen i koncentrationen af immunologiske biomarkører for det kirurgiske stressrespons (TNF-α, IL-6 og IL-10). De sekundære outcomes er ændringer i IL-8, IL-17A, C-reaktivt protein, hvide blodlegemer, cortisol, transferrin, ferritin og D-Dimer foruden lægemiddelkoncentrationen og antistoffer mod lægemidlet i blodbanen samt 30 dages postoperative komplikationer og længden af postoperativ ophold på hospitalet.

Resultater

Studie 1 viser, at præoperativ behandling med anti-TNF-α eller andre immunmodulerende lægemidler ikke har nogen indflydelse på anastomose-relaterede komplikationer. Det er imidlertid påfaldende, at andre studier om emnet kom frem til modsatrettede konklusioner med hensyn til behandling med anti-TNF-α lægemidler. Undersøgelsen bekræfter desuden tidligere fund; at corticosteroider og kolo-koliske anastomoser er associeret med postoperative komplikationer.

Studie 2 viser, at effekten af anti-TNF-α lægemidler på postoperative outcomes ifølge litteraturen varierer, men at TNF-α lægemidlerne øger risikoen for anastomotiske komplikationer i studier med lav risiko for bias. Flere meta-analyser om emnet er blevet publiceret med tvetydige resultater.

Studie 3 forklarer dette ved at vise, at meta-analyser, der inkluderer et stort antal patienter og anvender kvalitetsvurdering, viser en øget risiko for overordnede postoperative komplikationer hos patienter, der har modtaget behandling med anti-TNF- α lægemidler.

Studie 4 viser ikke nogen effekt af anti-TNF-α behandling på kirurgisk stressrespons. Undersøgelsen viser dog en stor variation i typen, dosen, behandlingsvarigheden, lægemiddelkoncentrationen og tilstedeværelse af anti-lægemiddelantistoffer hos patienter, der har modtaget anti-TNF-α lægemidler inden for 12 ugers før kirurgi. Dette kan måske forklare de modsatrettede konklusioner i de øvrige studier i den eksisterende faglitteratur.

Konklusion

Effekten af anti-TNF-α behandling på det postoperative resultat hos patienter med Crohns sygdom er en kombination af mange faktorer. Blandt de vigtigste er steroidbehandling, sygdomshyppighed, ernæringsstatus, rygning, præoperativ optimering, præoperativ lægemiddelkoncentration og tilstedeværelsen af antistoffer mod lægemidler. Derfor kan et behov for præoperativ pausering af anti-TNF-α ikke begrundes ud fra det eksisterende vidensgrundlag. Der er behov for en multi-center randomizeret kontrolleret undersøgelse for at bekræfte eller afvise dette.

This Ph.D. thesis is based on the following four papers:

1) El-Hussuna A, Andersen J, Bisgaard T, Jess P, Henriksen M, Oehlenschlager J, Thorlacius-Ussing O, Olaison G. **Biologic treatment or immunomodulation is not associated with postoperative anastomotic complications in abdominal surgery for Crohn's disease.** Scand J Gastroenterol. 2012; 47:662-8.

2) El-Hussuna, A. Krag A, Olaisson G,Bendtsen F, Gluud, LG. **The effect of anti-tumor necrosis factor agents on the postoperative complications in Crohn's disease patients undergoing abdominal operation: Systemic review and meta-analysis**. Dis Colon Rectum. 2013; 56:1423-33

3) El-Hussuna, A. Theede K, Olaisson G. **Increased risk of post-operative complications in patients with Crohn's disease treated with anti-tumour necrosis factor α agents: Systematic review.** Dan Med J. 2014;61:A4975

4) El-Hussuna, A., Qvist, N., Zangenberg, M.S, Langkilde, A., Siersma, V, Hjort, S, Gögenur, I. **No effect of anti-TNF-α agents on the surgical stress response in patients with inflammatory bowel diseases undergoing bowel resections: A prospective multi-centre pilot study** (submitted).

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Study 1

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Ph.D. project

1. Aalborg University Hospital, Department of Surgery
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ABBREVIATIONS

ADA	Adalimumab
Anti-TNF-α	Anti-tumour necrosis factor-α agent (biologic)
CD	Crohn's disease
CI	Confidence interval
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CZP	Certolizumab pegol
EBM	Evidence-based medicine
ECCO	European Crohn's and Colitis Organisation
ELISA	Enzyme-linked immunosorbent assay
ESCP	European Society of Coloproctology
GT	Gastrointestinal tract
HPAA	Hypothalamic-pituitary-adrenal axis
IASC	Intra-abdominal septic complications
IBD	Inflammatory bowel disease
IC	Indeterminate colitis
IFX	Infliximab
IgG	Immunoglobulin G
IL	Interleukin
IQR	Interquartile ratio
JAK/STAT	Janus kinase/signal transducer and activator of transcription
mAb	Monoclonal antibody
MAPK	Mitogen-activated protein kinase
mTNF-α	Trans-membrane TNF-alpha
NF-κB	Nuclear factor κ-B
NLR	Nucleotide-binding oligomerisation domain (Nod)-like receptor (NLR)
NOD	Nucleotide-binding oligomerisation domain
NSAID	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRR	Pattern recognition receptors
ROS	Reactive oxygen species
RR	Risk ratio
SSI	Surgical site infection
sTNF-α	Soluble TNF-alpha
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor-alpha
UC	Ulcerative colitis

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1. INTRODUCTION

1.1 CROHN'S DISEASE

1.1.1 DEFINITION, INCIDENCE AND PREVALENCE

Crohn's disease (CD) is a relapsing systemic inflammatory disease that mainly affects the gastrointestinal tract (GT). Extra-intestinal manifestations may present with or without the gastrointestinal symptoms.

The incidence of Crohn's disease is increasing and is currently estimated to be 6-15/100,000, whereas its prevalence is 50-200/100,000. The peak age of CD occurrence is 20-30 years, and the frequency of CD is 20-30% higher in women[1]. The disease shows a north-south gradient in terms of residence latitude, with a higher incidence of CD in northern compared with southern latitudes[2]. Recent studies have also shown an increased incidence of CD in previously low-risk countries, probably due to the adoption of a western lifestyle, as in the case of immigrants to western countries.

1.1.2 THE PATHOGENESIS OF CROHN'S DISEASE

CD is thought to result from an inappropriate inflammatory response to the gut microbial flora in genetically predisposed individuals[3,4].

The influence of *genetics* in CD has been shown in epidemiological studies that demonstrated different prevalences in different ethnic groups (higher risk of CD in Jews, particularly Ashkenazi Jews), familial aggregations (having a relative with CD increases the risk of developing CD) and concordance in twins (the rates of concordance in monozygotic and dizygotic twins are 30.3% and 3.6%, respectively). Genetic studies have identified 163 associated genes/loci, most of which are shared between CD and ulcerative colitis (UC); however, some of these genes/loci are also associated with other immune-related diseases[5]. Although inflammatory bowel disease (IBD) is currently classified into CD and UC, recent data suggest the existence of three genetically distinct groups of diseases (ileal CD, colonic CD and UC)[6]. However, CD and UC have no single highly penetrant genetic cause[7]. The identified genes/loci predispose individuals to CD by affecting the mechanism regulating the maintenance of GT homeostasis.

The maintenance of GT homeostasis is critically dependent on the capacity of the immune system to remain tolerant to harmless dietary and gut microbial flora-derived antigens while maintaining the ability to generate protective immune responses against harmful intestinal pathogens. This identification involves pattern recognition receptors (PRRs). The host possesses a variety of PRRs, including membrane-bound toll-like receptors (TLRs) and the cytoplasmic nucleotide-binding

oligomerisation domain (Nod)-like receptor (NLR). Genes/loci can affect these PRRs to induce the immune system to mount attacks on harmless commensal microorganisms.

The mechanism through which the *immune system* mounts these attacks remains under investigation but might be related to the stimulation of innate immune cells to produce proinflammatory cytokines and thereby facilitate further recruitment of innate immune cells. NOD signalling can also enhance the production of reactive oxygen species (ROS) by innate immune cells. Microbiota play an essential role in the reduction and oxidation states of the GT.

The composition of the *microbiota* and its interaction with the host can promote the development of IBD or trigger disease flares in patients with IBD. Environmental factors can trigger IBD in different ways, one of which involves changing the composition of the GT microbiota. How the gut microbiota and the immune system initiate the inflammatory process in genetically predisposed individuals is not yet fully understood. However, a three-stage model (**figure 1**) was proposed by Sewell et al. to explain the pathogenesis of CD[4]:

Stage 1: Penetration of luminal contents into the bowel wall, resulting in access to the underlying bowel tissues[4].

Stage 2: Impaired clearance of foreign material from the bowel wall, probably due to a defect in the secretion of proinflammatory cytokines by macrophages.

Stage 3: Compensatory adaptive immune responses that occur when the remaining uncleared debris is phagocytosed by macrophages, which leads to the formation of granulomata. Macrophage activation then results in a 'second wave' of secretion of proinflammatory cytokines and chemokines that drives the recruitment of T cells to the site and their polarization to the characteristic Th1 phenotype[4].

In healthy individuals, the T-cell population is regulated by apoptosis. Defective apoptosis thus seems to be a relevant pathogenic mechanism in CD. Most agents used for the treatment of IBD, including steroids, sulfasalazine, IFX, azathioprine, methotrexate, cyclosporine, tacrolimus and thalidomide, can induce the apoptosis of monocytes or lymphocytes. Different molecular apoptotic pathways appear to be activated by different drugs, and these pathways might explain the differential therapeutic effects of these agents[8].

Tumour necrosis factor alpha (TNF-α) plays an important role in all three stages described above and might explain the high TNF-α levels observed in patients with CD. In stage 1, TNF-α regulates the intestinal permeability by mediating tight junctions between intestinal epithelial cells[9]. It also regulates the secretion of mucin, which is crucial for the barrier function[10]. TNF-α is essential for the differentiation and proliferation of macrophages (stage 2) and plays a key role in the maturation, proliferation, differentiation and survival of T lymphocytes that occur in stage 3. Dysregulated TNF signalling might therefore cause aberrant immune T-cell function, leading to the onset of chronic inflammation. The structure and function of TNF-α are explained in the following section.

1.2 TNF-ALPHA

1.2.1 STRUCTURE AND MECHANISM OF ACTION OF TNF-ALPHA

TNF-α is a low-molecular-weight protein that mediates cell-to-cell communication. It is produced primarily by activated monocytes and macrophages in response to a variety of stimuli[11,12].

TNF-α is recognized by two receptors: TNF-α receptor-1 (TNF-αR-1), which is ubiquitously expressed in nucleated cells, and TNF-αR-2, which is expressed mainly in immune cells[11,12,13]. TNF-α is very potent, and an occupancy of its receptors as low as 5% produces a biochemical response[14,15]. After binding to its receptor, TNF-α activates a variety of intracellular signalling pathways that affect gene transcription[16]. TNF-α that is inserted into the cell membrane (mTNF-α) is cleaved by the matrix metalloprotease TNF-converting enzyme into a soluble form (sTNF-α). Both sTNF-α and mTNF-α are biologically active and can bind to and signal through TNF receptors[17,8,18].

TNF-α mediates apoptosis, survival, differentiation and proliferation through the activation of different pathways involving nuclear factor kappa B (NF-κB), Janus kinase/signal transducer and activator of transcription (JAK/STAT), p42/p44 mitogen-activated protein kinase (MAPK) and p38 MAPK (**figure 2**). The activation of specific signalling pathways depends on multiple costimulatory intra/extracellular factors [11,12,13,19]. In addition to its action on effector cells (forward signalling), mTNF-α also functions as a cellular receptor in TNF-α-producing cells, resulting in "reverse signalling". Through this process, the membrane-integrated ligands can receive signals, acting as receptors that transmit positive and negative feedback signals to the ligand-bearing cell[17,20,21]. TNF-α, which is barely detectable in quiescent cells, orchestrates inflammatory processes by switching on a TNF-dependent cytokine cascade in an autocrine and paracrine manner[17]. This activation leads to a variety of effects that are examined in detail in the next section.

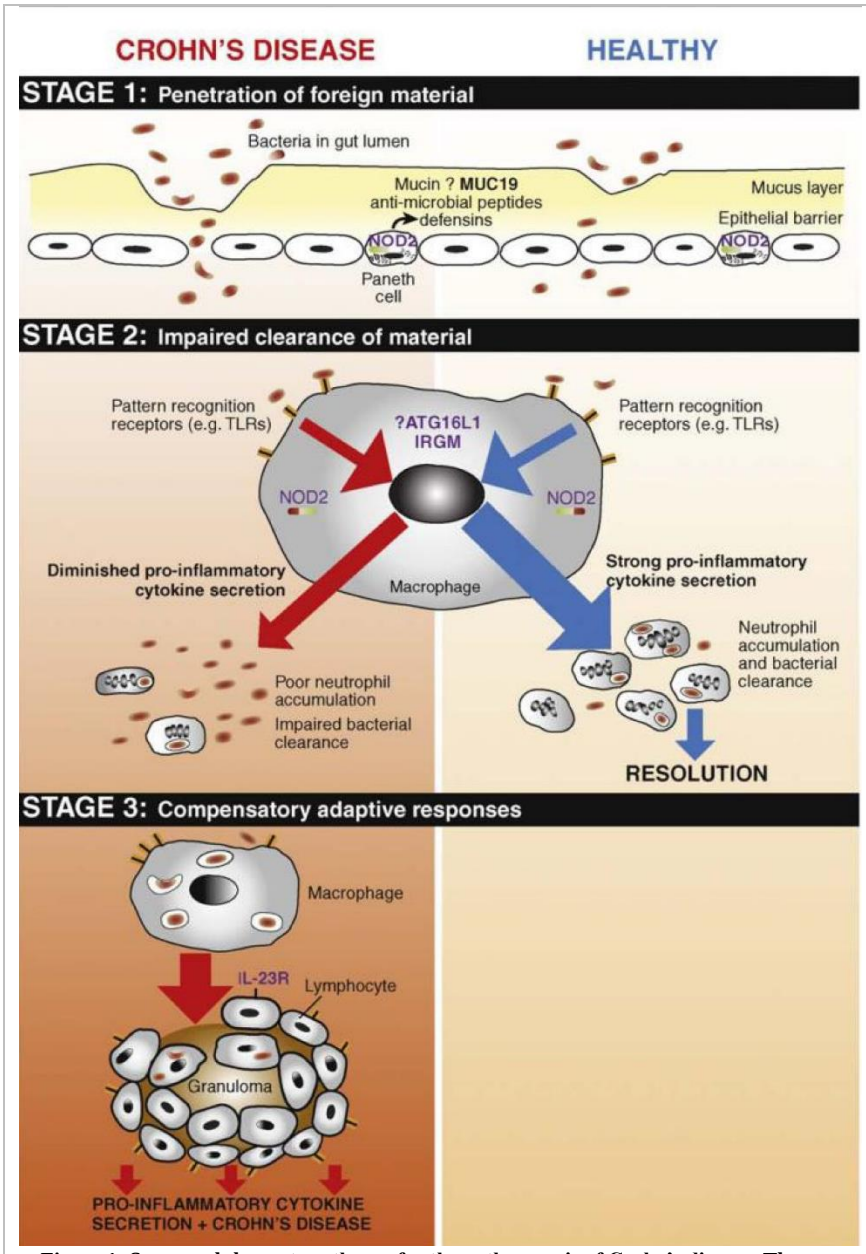

Figure 1: Suggested three-stage theory for the pathogenesis of Crohn's disease. The first stage is initiated by penetration of the luminal contents into the bowel wall due to defects in tight junctions. In the second stage, impaired clearance of foreign material from the bowel wall leads to compensatory adaptive immune responses and the formation of granulomas, triggering the secretion of proinflammatory cytokines in stage three. From Sewell 2009, Current Opinion in Immunology (with permission from Elsevier).

1.2.2 PHYSIOLOGICAL EFFECTS AND FUNCTIONS OF TNF-ALPHA

TNF-α stimulates the hypothalamic-pituitary-adrenal axis (HPAA), and this stimulation induces the release of corticotropin-releasing hormone (CRH), followed by the secretion of steroids that regulate many bodily functions, including the stress response. The secretion of C-reactive protein (CRP) by hepatocytes, the chemotaxis of neutrophils and the regulation of dendritic cell and macrophage functions are only a few of the known functions of TNF-α[12]. The dose-related role of TNF-α in wound healing[19,22,23,24] is crucial and will therefore be explained in detail.

Disruption of the protective skin barrier activates an orchestrated cascade of events involving growth factors, cytokines and chemokines, leading to wound healing through a process that can be divided into three overlapping phases:

(i) Inflammatory phase: TNF-α stimulates neutrophil and macrophage activity and recruitment to the site of injury (chemotaxis)[25,26,27]. It also stimulates the production of other cytokines, such as IL-1 and IL-6, both of which are important during the wound healing process[28].
(ii) Proliferative phase: The process of angiogenesis, proliferation and re-epithelialization is triggered by ROS released secondary to damage to the blood vessels[25]. TNF-α is essential in this process.
(iii) Tissue remodelling: TNF-α can inhibit or stimulate collagen synthesis by fibroblasts. The effect depends on the concentration of TNF-α in the tissue[26,28,29].
Physiological release of TNF-α following injury is therefore essential for proper healing and timely recovery[24,29–31]. In contrast, the inhibition of TNF-α is essential for preventing the effects of overproduction, and this inhibition can be achieved via various regulatory feedback mechanisms, as follows[32]:
1. sTNFR: Soluble TNF-α receptors compete with cellular receptors for the binding of free TNF-α in response to excessive systemic TNF-α activity[15,19].
2. Glucocorticoids: Glucocorticoids were the first identified inhibitors of TNF-α production.
3. Prostaglandin E2: This lipid, which is produced during inflammation, effectively switches off TNF-α synthesis.
4. IL-10 and the IL-10 family: This family consists of the prototypic "anti-inflammatory cytokines" that inhibit TNF production in vitro and in vivo.
5. Cholinergic anti-inflammatory pathway: The inhibition of TNF synthesis is mediated by acetylcholine acting on macrophages.

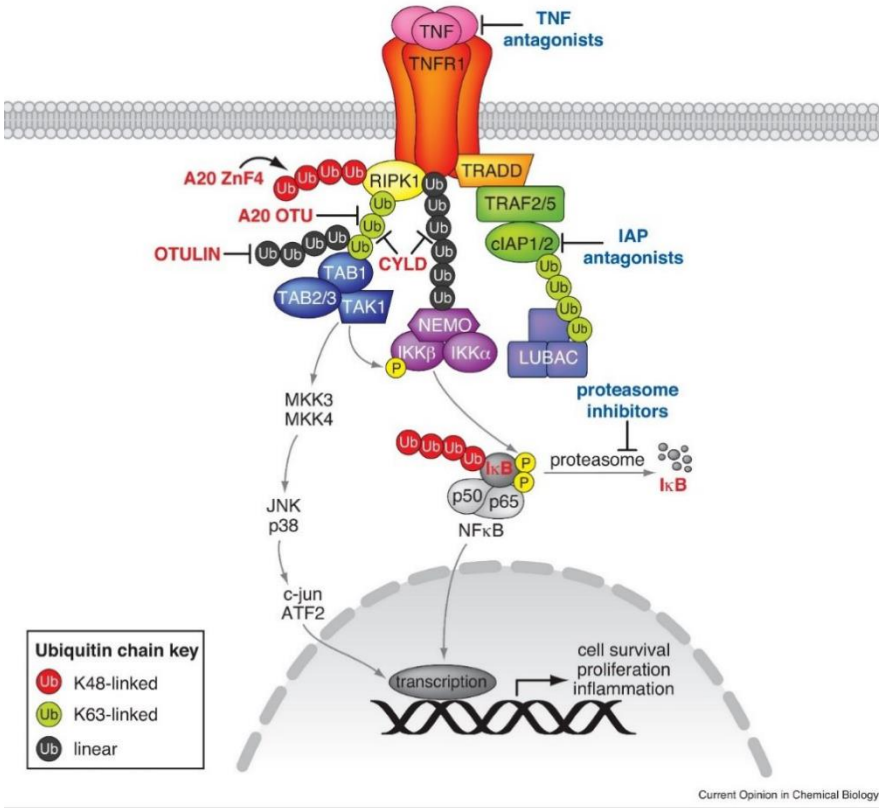

Figure 2: Activation of different pathways by TNF-α upon binding to TNF-α receptors. The propensity for the activation of each pathway depends on multiple co-stimulatory intra- and extracellular factors. NF-κB: nuclear factor kappa B; JAK/STAT: Janus kinase/signal transducer and activator of transcription; MAPK: mitogen-activated protein kinase. From Wertz 2014, Current Opinion in Chemical Biology (with permission from Elsevier).

Patients with CD who have high TNF-α levels in their lamina propria might exhibit a disturbance of feedback regulatory mechanisms. TNF-α is believed to play a role in the initiation and amplification of the mucosal inflammatory cascade in CD[20,33,3435]. Microbial products constantly stimulate the production of TNF-α by triggering TLRs and NOD-2 PRRs. Depending on the dose and the presence of secondary signals, TNF-α can induce either protective or destructive effects on tight junctions[36]. Leaky junctions permit the access of microbial products to underlying intestinal tissue. This exposure initiates an inflammatory cascade that is regulated by many mediators, one of which is TNF-α, which exerts its action through T-cell apoptosis. The blockage of TNF-α can halt or even reverse this process, as discussed in the next section.

1.3. ANTI-TNF-ALPHA AGENTS

1.3.1 TREATMENT STRATEGIES

Inactivation of TNF-α can be achieved in two ways: the use of monoclonal antibodies (mAbs) (for instance, IFX) that bind to TNF-α, and the use of fusion proteins that bind to TNF receptors (for instance, Etanercept). Both methods inactivate TNF-α and prevent TNF-α-mediated inflammatory processes. The use of anti-TNF-α has changed the classical approach for the treatment of CD, which aimed to manage disease flare-ups using immunosuppressive agents. Anti-TNF-α might result in complete remission of the inflammatory state, avoiding the use of steroids, which would improve the patient's quality of life and work productivity and reduce the need for hospitalization and surgery[37,38]. However, many population-based studies have not shown this effect[39–41].

The conventional approach for the management of active CD is based on the progressive intensification of therapy ("step-up") as the disease worsens: this therapy starts with 5-ASA, steroids, and immunomodulators, and the use of anti-TNF-α is reserved for severe or non-responding cases. However, there is a growing consensus that the ultimate goal of CD management must be complete disease control and not merely clinical improvement. Based on this goal, key therapeutic outcomes have moved beyond the control of clinical symptoms to include steroid-free remission and mucosal healing (endoscopic remission), which usually leads to a significantly better clinical outcome and a significantly improved quality of life[42]. A top-down approach to medical therapy is increasingly being adopted for patients with risk factors for severe inflammation or an unfavourable disease course in an attempt to halt the inflammatory process at the earliest possible stage. Patients with mild-to-moderate inflammation and fewer risk factors may benefit from an accelerated step-up approach. Different types of anti-TNF-α are used in the above-mentioned approaches, these drugs can be switched in the case of a lack of response or allergic reaction. The structure and pharmacokinetics of the currently available anti-TNF-α agents are discussed in the next section.

1.3.2 STRUCTURE AND PHARMACOKINETICS

Four anti-TNF-α biological agents are currently approved for the treatment of IBD (**figure 3**). Infliximab (IFX) (Remicade® and its two biosimilars, Inflectra® and Remsima®), adalimumab (ADA) (Humira®) and golimumab (Simponi®) are bivalent monoclonal antibodies, and certolizumab pegol (CZP) (Cimzia®) is a PEGylated monovalent Fab antibody fragment[16]. The drug structures are illustrated in (**figure 3**).

IFX (Remicade®) is a chimeric mAb comprising human and murine variable regions, and this mAb is administered via the intravenous (IV) route. The use of this route allows the administration of large volumes and results in a more rapid central distribution with a low variability in bioavailability compared with subcutaneous

(SC) administration, which might be associated with some problems. The induction and maintenance dose of IFX for both UC and CD patients is 5 mg/kg every 8 weeks.

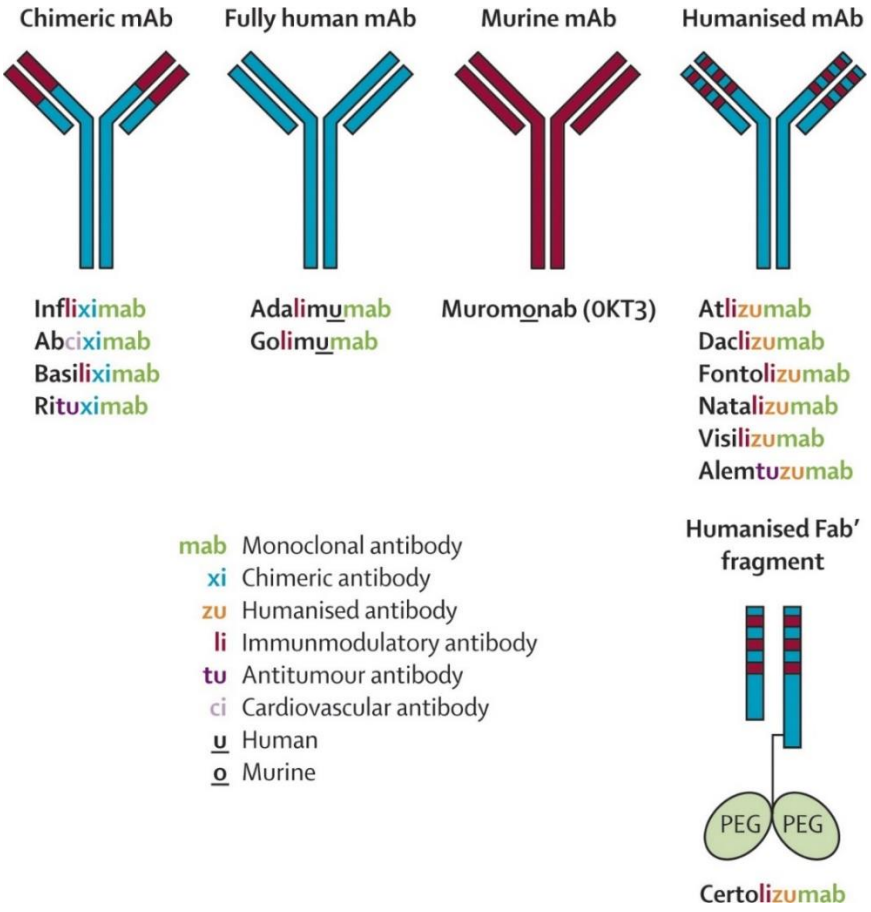

Figure 3: Structures of anti-TNF-α agents. The figure illustrates the labelling of these monoclonal antibodies according to the human and murine variables in their structures. From Baumgart 2007, the Lancet (with permission from Elsevier).

ADA (Humira®) is a human IgG1 mAb that was derived through phage display and is administered via the SC route at a dose of 40 mg once every 2 weeks for induction and maintenance in both UC and CD[43].

Golimumab (Simponi®) is a mAb derived from transgenic mice engineered to express human IgG and is administered subcutaneously at a dose of 50 mg or 100 mg once every 4 weeks in UC and CD[43].

CZP (Cimzia®) is a monovalent Fab antibody fragment that was covalently linked to two cross-linked chains of polyethylene glycol to increase its half-life in vivo, enhance its solubility and possibly reduce its immunogenicity. Cimzia® is

administered via the SC route at 400 mg once every 4 weeks for induction and maintenance in CD[16, 21,20,43].

Due to their large size, the renal clearance of these antibodies is almost non-existent. The drugs' half-life is approximately 2 weeks[43]. The direct comparison of these antibodies revealed that IFX, ADA, and CZP display comparable mTNF-α antagonist activity in vitro[20].

The pharmacokinetic profile of anti-TNF-α agents is influenced by many patient-, disease- and drug-specific factors, including body weight, immunogenicity, the use of concomitant immunosuppressive medication (for instance, combining the therapy with methotrexate delayed the decrease in the serum concentrations of IFX[44]) and drug loss through a 'leaky gut'. These inter- and intra-individual differences contribute to the problem of an inadequate response and a loss of response[16]. In some studies, no anti-TNF-α was detectable in some patients 12 weeks after administration of the last dose[44], which might explain why 12 weeks prior to the operation was selected as the therapeutic window in almost all studies of the effect of anti-TNF-α on postoperative complications[45].

1.3.3 MECHANISM OF ACTION OF ANTI-TNF-ALPHA

The efficacy of anti-TNF-α antibodies in IBD has been attributed to several effects, but their precise molecular mechanisms of action have not been thoroughly characterized[20,35]. Compared with rheumatoid arthritis, a disease in which most TNF-blocking strategies seem to be therapeutic, some mechanisms other than the neutralization of sTNF-α are involved in the resolution of inflammation and mucosal healing in CD[8,20,21,46]. The induction of lamina propria T cell apoptosis and reverse signalling, which leads to the downregulation of cytokine production and apoptosis,[20,21] have been described as an alternative mechanism of action of anti-TNF-α agents in vitro and in vivo.

Apoptosis (**figure 4**) is a form of programmed cell death. This natural physiological process is essential for normal development and the maintenance of cell homeostasis. In contrast to necrosis, which is a form of traumatic cell death, apoptosis allows the 'silent' elimination of unwanted cells. A defect in the apoptosis of T cell populations in the lamina propria is an important factor in the pathogenesis of IBD[17,8]. The use of endoscopy and technetium-labelled annexin V has shown that T cell apoptosis in the lamina propria occurs within 24 hours after the administration of IFX[46].

Bax and Bak are two nucleus-encoded proteins present in higher eukaryotes; these proteins can pierce the mitochondrial outer membrane and thereby mediate cell death by apoptosis. IFX and ADA induce apoptosis by upregulating the expression of Bax and Bak and thereby increasing the release of cytochrome C from the mitochondria into the cytosol[17]. Another alternative pathway leading to apoptosis is regulated by specific death receptors that induce the formation of a death-inducing signalling complex. These two pathways are illustrated in **figure 4**. Like all drugs, anti-TNF-α agents are associated with side effects, as discussed below.

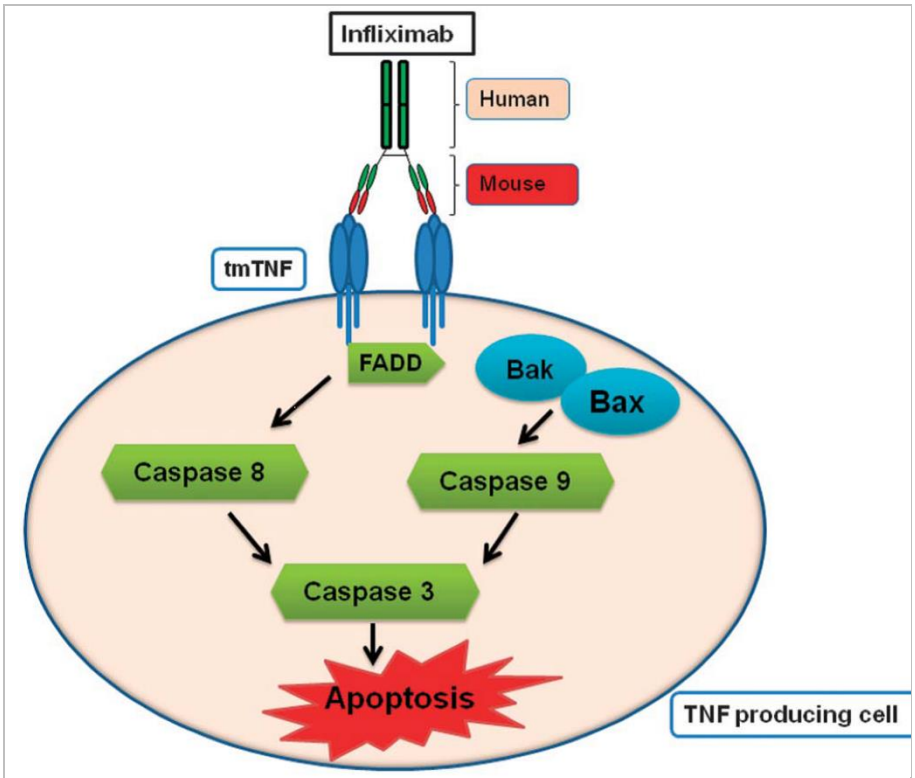

Figure 4: Action of anti-TNF-α agents via apoptosis, which is a form of programmed cell death that is essential for normal development and the maintenance of cell homeostasis. A defect in the apoptosis of mucosal T cell populations might be important in the pathogenesis of IBD. Bax and Bak are two nuclear-encoded proteins that penetrate the mitochondrial outer membrane and mediate cell death by apoptosis. Caspase-3 is a key effector of apoptosis. FADD: Fas-associated death domain; tmTNF: transmembrane TNF. From Slevin 2015, Inflamm. Bowel Dis. (with permission from Wolters Kluwer Health, Inc.).

1.3.4 SIDE EFFECTS OF ANTI-TNF-ALPHA

Serious infections are caused either by latent disease reactivation or by *de novo* disease acquisition. Opportunistic infections include viral infections (e.g., cytomegalovirus), bacterial infections (e.g., tuberculosis), and fungal infections (e.g., candidiasis). To prevent the development of infectious diseases during immunosuppressive treatment, effective vaccines should be considered prior to initiation of an anti-TNF-α therapy. Careful scrutiny for tuberculosis using chest X-rays and QuantiFERON® testing is mandatory, and screening using HIV tests and hepatitis tests should also be performed prior to starting treatment. Potential malignancies (e.g., lymphoma) and paradoxical inflammation of the skin and other

organs, which might lead to paradoxical psoriasis and lupus-like drug reactions, have also been reported as serious side effects.

1.3.5 SURGERY AND RISKS OF POSTOPERATIVE COMPLICATIONS

For decades, surgery for CD was an option only in cases involving failed medical treatment. This concept was associated with a high rate of emergency surgeries, increased risk of short bowel syndrome, higher risk of postoperative morbidity and mortality and low quality of life. Fortunately, this concept has been challenged in the last few years, and surgery has become an option for patients with CD at various stages of the disease. A recent randomized controlled trial (RCT) showed that the results of laparoscopic ileocaecal resection with respect to quality of life are comparable to those of treatment with anti-TNF-α[47]. Rapid developments in surgery, including bowel-sparing surgery, minimally invasive access to the abdomen, better timing of surgical intervention[48] and better preoperative optimization[49,50], have made surgery a decent choice for the treatment of CD. In most tertiary centres, IBD surgeons are members of multidisciplinary teams that are responsible for treating patients with CD. This collaboration might decrease the rate of emergency interventions and increase the chance of multi-modal preoperative optimization, thus reducing the rate of postoperative morbidity[49].

A recent study showed that patients need balanced information on all treatment options, including surgery, from a dedicated multidisciplinary team beginning at the early stage of their disease[51]. Thus, patients should meet with an IBD surgeon early in their treatment pathway and not just when major surgery has become inevitable. There are three types of surgery for CD:

1. **Stoma formation** might be indicated when anastomosis is not advisable, as in cases of severe inflammation, intra-abdominal abscess, severe small bowel obstruction or large bowel ileus (megacolon). It is occasionally required for severe unrelenting perianal CD. Patients with CD may worry about stoma-forming surgery and sometimes endure a poor quality of life to avoid this surgery[51]. Early multidisciplinary counselling about stoma may help patients with their decision-making process.

2. In **strictureplasty**, the affected bowel segment is incised and re-sutured without resection. A variety of methods have been described for strictureplasty (Heinke-Mikulics, Finney and Michelassi's isoperistaltic side-to-side strictureplasty). Some researchers are beginning to question whether strictureplasty alone, rather than formal resection of the diseased bowel, may suffice. Recent studies have demonstrated full mucosal healing at the strictureplasty site[52,53]. These experimental ideas, if proven, might change the management of patients with CD, resulting in surgery being performed much earlier.

3. **Resection of the affected bowel segment** with primary anastomosis is the most frequent operation in CD[51]. A variety of techniques have been described for performing anastomosis, and these vary regarding the configuration (side-to-side, end-to-side, or end-to-end anastomoses), method (stapling or hand-sewn suture)[54] and technique (extra-or intra-corporal anastomosis). There is no overriding consensus regarding the optimal technique for intestinal anastomosis in CD. The choice of method must therefore be tailored to the individual patient.

Risk of surgery

A meta-analysis of 26 published population-based studies calculated the risks of surgery at 1, 5, and 10 years after a diagnosis of CD as 16.3%, 33.3%, and 46.6%, respectively[55]. However, a recent epidemiological study conducted in Europe showed that only 22% of patients with CD underwent resection during 5 years of follow-up, and 13% of these resections were performed in the first year after the diagnosis. The median time to first surgery was 7 months (IQR 1-30) and did not differ between patients in Western and Eastern Europe[56]. It is unclear whether the rate of surgery decreased or remained unchanged after the introduction of anti-TNF-α agents[57]. Population-based studies have yielded conflicting results regarding the impact of anti-TNF-α agents on the reduction of surgical intervention in patients with CD[58,38,59,60]. The above-mentioned meta-analysis concluded that the risks of surgery 1, 5 and 10 years after a diagnosis of CD have decreased during the last six decades[55]. Data from referral centres' cohorts have shown that up to 50% of patients are exposed to anti-TNF-α agents prior to their first surgery[61,62], and this exposure rate has led some researchers to suspect a risk of postoperative complications in patients treated with anti-TNF-α agents.

This suspicion is based on the knowledge that TNF-α is an important component of the body's immune defence against infections in addition to being essential for wound healing through its effects on angiogenesis[25] and collagen synthesis[23,24,30]. The inhibition of these pathways might impair wound healing after surgery and increase the risk of postoperative complications, such as surgical site infection and anastomosis-related complications, in patients with CD. This possibility has been investigated and debated in more than 60 studies, and the conclusions reached by the authors of these studies are divergent, leading to different policies regarding the preoperative withdrawal of anti-TNF-α. Surgery is still needed in the treatment of CD, but whether early surgery can alter the natural course of the disease is unclear.

2. OVERALL AIM AND HYPOTHESIS

The aim of this thesis was to investigate the effect of anti-TNF-α agents on the postoperative outcome in patients with CD undergoing bowel resection. The hypothesis in question was investigated through four studies.

3. METHODS

3.1 STUDY 1

3.1.1 HYPOTHESIS AND OBJECTIVES

Anti-TNF-α agents increase the risk of postoperative complications in patients with CD undergoing bowel resection.

3.1.2 STUDY DESIGN

The study was designed as a retrospective cohort multi-centre study using data collected from hospital registries.

3.1.3 PRIMARY AND SECONDARY OUTCOME VARIABLES

The primary outcome was intra-abdominal septic complications (IASCs) defined as overt anastomotic dehiscence, postoperative enteric fistula, or intra-abdominal abscess requiring treatment with laparotomy, laparoscopy or percutaneous radiologically guided drainage. The secondary outcome variables were "other postoperative septic complication" and "any complication."

3.1.4 DATA COLLECTION

The study included operations for CD performed at four Danish university hospitals during the period 2000-2007. All operations involving resection and anastomosis and/or strictureplasty were included in the study. The diagnosis of the operated patients was based on operative and histopathological findings, and that of previously non-operated patients was based on endoscopic, radiological, and clinical findings.
Demographic characteristics, histories of disease and of medical and surgical treatments, in addition the timing of the surgical intervention (acute, emergent or elective) were collected. A patient was considered to have had anti-TNF-α treatment if s/he had received IFX, ADA, CZP or golimumab within 3 months prior to the operation. A patient was considered to be on immunomodulation if s/he was treated with azathioprine, 6-mercaptopurine, or methotrexate for more than 3 months and received the last drug dose within 1 month prior to the operation. A patient was considered to be on steroids if s/he was treated for more than 4 weeks and received the last drug dose within 1 week before surgery. A dose of prednisolone of at least 20 mg was considered a high dose. Operations performed within 24 hours of admission were considered acute, those performed within 1 week of admission were defined as emergent, and planned operations were defined as elective. During the postoperative course, any complication, readmission, and death within 30 days were registered.

3.1.5 STATISTICAL ANALYSIS

Univariate comparisons were performed using Pearson's chi-square test or Fisher's exact test when appropriate. Continuous data were analysed using the Mann-Whitney U-test. A multivariate logistic regression analysis was employed to identify independent predictors of outcome. Continuous variables were dichotomized at their median values, and the median values were used in the binary logistic regression analyses. Three dependent variables were tested by the model: IASCs, septic complications, and "any complication". As covariates, the model included age, gender, and variables that showed significant differences in the univariate analysis. A two-sided p value less than 0.05 was considered to indicate significance. The statistical analysis was performed by the first and corresponding author with help from a statistician (Hvidovre Hospital, research section).

3.2 STUDY 2

3.2.1 HYPOTHESIS AND OBJECTIVES

Anti-TNF-α agents might increase the risk of postoperative complications due to their mechanism of action. It was hypothesized that pooling the data from all the published studies on the subject, sorting the studies according to their risk of bias and then analysing their results would lead to statistically powerful and clinically useful conclusions.

3.2.2 STUDY DESIGN

The study involved a systematic review and meta-analysis based on a predefined study protocol that was developed according to the recommendations in the Cochrane Handbook for Reviews of Interventions and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[63].

3.2.3 PRIMARY AND SECONDARY OUTCOME VARIABLES

The primary outcome measure was anastomotic complications including overt anastomotic leak (dehiscence), postoperative intra-abdominal abscess or enteric fistula verified by diagnostic imaging, laparotomy or laparoscopy. The secondary outcome measures were the total numbers of complications, non-anastomotic surgical complications, infectious complications, major medical complications, and minor medical complications, reoperation, and mortality. Medical complications were classified as major if they were potentially life-threatening or prolonged the hospital stay. The remaining medical complications were classified as minor. The outcome measures were assessed after 30 days of follow-up.

3.2.4 DATA COLLECTION

Studies were identified through electronic and manual searches. The electronic searches were performed in MEDLINE, EMBASE, and the Cochrane Library, and all

databases were searched from their date of inception to September 2012. The search strategy for MEDLINE is included in manuscript II.

Case control and cohort studies were included irrespective of the publication status, year of publication, and language. No language filter was applied; however, only English-language studies were found. The included studies assessed patients with CD undergoing laparoscopic or open abdominal surgery. To ensure the completeness of the evidence, studies of CD patients alone as well as studies of mixed IBD populations (CD and UC) were included. Based on the pharmacokinetic studies, the intervention group included patients who received any type or dose of anti-TNF agent within the 3 months prior to surgery[44], and patients who did not receive anti-TNF-α agents were included in the control group.

One author (A.E.) performed the literature searches and listed the eligible studies. A research librarian at Copenhagen University library repeated the search to ensure the identification of all published studies.

The excluded studies were listed, and the reasons for exclusion were reported. All the authors participated in the final selection of the included studies. Three authors (A.E., A.K., and L.G.) independently extracted the patient data, interventions, study characteristics and all outcome measures. Differences were resolved by discussion. The authors of the included studies were contacted for additional information when necessary.

3.2.5 STATISTICAL ANALYSIS

The quality of bias control in the included studies was assessed using the Newcastle-Ottawa Scale[64]. Each study was assigned a number of stars based on the selection of patients for the intervention and control groups (maximum 4 stars), the comparability of the intervention and control groups (maximum 2 stars), and the ascertainment of the outcome of interest (maximum 3 stars). A lower number of stars indicates a greater risk of bias.

The primary analyses were performed through meta-analyses using random-effects models due to an expected clinical heterogeneity. The results are reported as risk ratios (RRs) with 95% confidence intervals. Heterogeneity was assessed using the I^2 statistic (the fraction of variance due to heterogeneity), and the resulting values classified (according to the Cochrane handbook with modification) the heterogeneity as unimportant (I^2 < 30%), moderate (I^2 30%-50%), substantial (I^2 51%-75%), or considerable (I^2 > 75%). Sensitivity analyses were performed by repeating the meta-analyses on the odds ratio (OR) scale and using fixed-effects models. In addition, sensitivity analyses excluding the studies that included mixed populations of patients (CD and UC) were performed to test the influence of these studies on the results. The results of the sensitivity analyses are only reported if the conclusions differed from those reached in the primary meta-analyses. Regression analyses and the Egger test (a statistical test for the assessment of funnel plot asymmetry) were performed to evaluate the risk of small-study effects (including publication bias). Subgroup analyses were performed to evaluate the influence of bias control and publication status (full article or abstract). Differences between subgroups were evaluated using

the test for subgroup differences, and the results are presented as *p* values. An estimated 78 patients are needed to show a 20% difference in complications (with α set to 5% and power set to 80%). The analyses were performed with help from Liselotte Glud using RevMan version 5.0.5 (Nordic Cochrane Centre, Copenhagen, Denmark) and STATA (version 12, StataCorp, TX, USA).

3.3 STUDY 3

3.3.1 HYPOTHESIS AND OBJECTIVES

The results of 18 retrospective studies regarding the effect of anti-TNF-α on postoperative outcome were conflicting, and similar findings were obtained from the meta-analyses based on these studies. The objective of this study was to review the data from the published reviews and meta-analyses to reach definitive conclusions concerning the impact of anti-TNF-α treatment on postoperative outcome.

3.3.2 STUDY DESIGN

This was a narrative systematic review. The literature search performed in this systematic review was the same as that used in study 2. The review was conducted based on a predefined study protocol that was developed according to the recommendations presented in the Cochrane Handbook for Reviews of Interventions and the PRISMA statement.

3.3.3 PRIMARY OUTCOME VARIABLE

The primary outcome measure was 30-day postoperative complications, with a particular focus on anastomotic leaks.

3.3.4 DATA COLLECTION

Studies were identified through electronic and manual searches. The electronic searches were performed in MEDLINE, EMBASE, and the Cochrane Library, and all databases were searched from their date of inception to September 2012. The search strategy for MEDLINE is described in manuscript III.

Reviews or meta-analyses were included, and no filters were applied during the search. All reviews that assessed patients with CD undergoing laparoscopic or open abdominal surgery were included. One author (A.E.) performed the literature searches and listed the eligible studies. The search was then independently repeated by a research librarian at the University of Copenhagen Library to ensure that no relevant studies had been missed. All the authors participated in the final selection of the included studies, and differences were resolved by discussion.

3.3.5 STATISTICAL ANALYSIS

This study was a narrative systematic review without statistical analyses. The six meta-analyses included in the review had many methodological differences. They employed different inclusion criteria (for instance, inclusion of studies with mixed

CD and UC populations, inclusion of abstracts, inclusion of low-quality studies and of studies exploring only one or several anti-TNF-α agents), different outcome measures (for instance, anastomotic leak, overall complications or septic complications) and different methods for reporting outcomes (for instance, minor a versus major complications or medical versus surgical complications). Therefore, statistical analysis was not conducted in study 3.

3.4 STUDY 4

3.4.1 HYPOTHESIS AND OBJECTIVES

In addition to being an important actor in immune defence, TNF-α plays a role in angiogenesis and collagen synthesis. Both processes are initiated by the stress response, and the inhibition of these pathways by anti-TNF-α agents might increase the risk of infections and impair wound healing in IBD patients after surgery. The aim of this study was to describe the surgical stress response in IBD patients and to investigate whether anti-TNF-α agents modify the surgical stress response and thus impact the postoperative outcome.

3.4.2 STUDY DESIGN

This was a prospective, non-interventional multi-centre pilot study with the following inclusion criteria: adult patients with CD or UC who were scheduled to undergo elective intestinal resection or terminal stoma closure in three Danish tertiary centres during the study period (March 2014-May 2016). Open and laparoscopic approaches were included. The exclusion criteria included patients with preoperative sepsis, patients with acute intestinal obstruction, patients who underwent an acute operation (within 48 hours of admission) and patients who underwent loop ileostomy takedown without laparotomy or laparoscopy.

3.4.3 PRIMARY AND SECONDARY OUTCOME VARIABLES

The primary outcome measure was the difference in the plasma concentrations of the main immunological biomarkers of the surgical stress response (TNF-α, IL-6, and IL-10) in anti-TNF-α-treated and anti-TNF-α-naïve patients.
The secondary outcome measures were differences in the plasma concentrations of other biomarkers of surgical stress, including IL-8, IL-17A, the TNF-α/IL-10 and IL-6/IL-10 ratios, the concentration of cortisol, transferrin, ferritin, and D-dimer. in addition to 30-day postoperative complications as defined in the study protocol, and postoperative length of stay at hospital (LOS).
Overall complications were defined as any deviation from the expected postoperative recovery. IASCs were defined as overt anastomotic leakages, intra-abdominal abscess formation or enteric fistula, and superficial surgical site infection (SSI) was defined as clinically documented skin infection at the site of surgery with or without positive

culture. The grade of the complications was assessed using the Clavien-Dindo classification of surgical complications.

3.4.4 DATA COLLECTION

Laboratory procedures

The choice of sampling intervals of 6, 24 and 48 hours after surgical incision was based on previous studies[11,65,66]. Biomarkers of surgical stress were selected according to the existing evidence[11,25,71–76,27,30,31,65,67–70]. Peripheral blood samples were obtained prior to the induction of anaesthesia and 6, 24 and 48 hours after surgical incision. EDTA-treated plasma and serum were separated by centrifugation, aliquoted and stored at -80°C until analysis.

The concentrations of anti-TNF-α agents in peripheral blood (drug concentration) and of antibodies against the specific compound used in the treatment (anti-drug antibodies) were measured on the day of surgery. The details of the methods used are explained on the laboratory homepage[77].

Cortisol was measured by ELISA (catalogue number EIA 1887, DRG International, Inc., Marburg, Germany). IL-6, IL-10, IL-17A, and TNF-α were measured using a human high-sensitivity magnetic ProCartaPlex Luminex kit (catalogue number EPX040-00000-801, eBioscience, Vienna, Austria). IL-8 and D-dimer were measured using ProCartaPlex Human IL-8 Simplex, ProCartaPlex Human D-Dimer Simplex, and Human Basic kits (catalogue numbers EPX010-10204-901, EPX010-12149-901, and EPX010-10420-901, eBioscience, Vienna, Austria). All samples were measured in duplicate according to the manufacturer's instructions, and the means of the obtained values were used in the statistical analyses. The plasma levels of CRP, transferrin, ferritin and D-dimer were measured by the Department of Clinical Biochemistry of Copenhagen University Hospital, Amager and Hvidovre, Denmark, using standard methods.

Anaesthesia, surgery and postoperative care

The incision time in all the included operations was approximately between 08:00 a.m. and 12:00 p.m. to eliminate/minimize circadian rhythm variation as a confounder. General anaesthesia was administered according to the standard practice of the anaesthesia department in the participating hospitals with modifications according to the study protocol. These modifications included the avoidance of steroids, NSAIDs and epidural analgesia. All patients received a single dose of prophylactic preoperative antibiotics at the induction of anaesthesia. The type and dose of anaesthesia were determined by the local standard of preoperative care in the participating hospitals. Laparoscopic surgery and enhanced postoperative recovery principles were the standard procedures in the participating centres.

Ethical considerations

The study was approved by the Ethics Committee in the capital region (reference number H-2-2013-166) and the region of Zealand (SJ-399) and by the Danish Data Protection Agency (Datatilsynet) in the capital region (reference number HVH-2013-

046 / 02515) and the region of Zealand (reference number REG-85-2013). The study was registered at clinicaltrials.gov (Identifier: NCT01974869) and Trial map on the ESCP website (http://www.escp.eu.com/research/international-trials/trials-map). This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

3.4.5 STATISTICAL ANALYSES

Sample size

The reference values for the changes in biomarkers for surgical stress in IBD patients that were needed for precise sample size calculation were not available at the time of the study. Chalhoub et al. showed that 28 patients were needed to demonstrate a significant change in TNF-α concentration after moderately stressful surgery[78]. Dimopoulou et al.[69] detected a significant correlation between TNF-α concentration and postoperative complications using 40 patients. Both of the foregoing studies were conducted on non-IBD patients but used settings similar to those used in study 4. Based on these two studies and on a meta-analyses by the authors[79], the study was a priori designed to recruit at least 40 patients, 1/3 of whom had received anti-TNF-α treatment prior to surgery.

Statistical methods

The preoperative and perioperative characteristics of the anti-TNF-α-treated and anti-TNF-α-naive patients were compared using the chi-squared test. Median, interquartile range (IQR), and minimum and maximum values were used to compare the changes in the concentration of biomarkers from baseline (before operation) to each of the postoperative follow-up time points (6, 24 and 48 hours after surgical incision). The differences in the median values of the changes from baseline in the anti-TNF-α-treated and anti-TNF-α-naive patients were assessed using a bootstrap approach. To reduce bias from confounding factors, the calculated medians were weighted using a propensity score, i.e., the inverse of the estimated probability of the administered anti-TNF-α regimen conditional on potential confounders. These probabilities were estimated using a multivariable logistic regression model that included the Harvey-Bradshaw index of disease severity, a nutritional risk screening score, whether parenteral nutrition was used, previous IBD-related abdominal operations, steroid stress dose (high dose of steroids with induction of anaesthesia in patients who received this treatment or who received steroids in the preoperative period), preoperative dexamethasone, epidural analgesia, method of access to the abdominal cavity (open versus laparoscopic), type of resection and disease classification in cases of CD. These factors were either significantly different among the patients who underwent the two anti-TNF-α regimens or were deemed to be important determinants based on clinical experience. Propensity scores were recalculated within each bootstrap replicate.

Logistic regression was used to investigate the postoperative outcome, and adjustment for confounding factors was performed by stepwise backwards elimination beginning with a model that included all pre- and perioperative characteristics that were deemed

to be clinically and/or statistically significantly different between the two treatment groups. The variables were then removed one by one until all variables had a p value < 0.10.

All analyses except the bootstrap analyses were performed using IBM SPSS Statistics for Windows, Version 19.0, IBM Corp. 2010 (Armonk, NY, USA). The bootstrap analyses were performed in the R environment for statistical computing version 3.1.2. A significance level of 5% was chosen.

4. RESULTS

4.1.1 RESULTS OF STUDY 1

The study included 417 operations on 369 patients (249 women); the median age at operation was 37 years (range 8-90 years). Thirty-two patients received preoperative anti-TNF-α treatment. There were no differences in the demographics or preoperative characteristics of the patients between the two groups (**Table 1**).
IASCs occurred in 13% (52/417) of the operations. Of these complications, overt anastomotic leak occurred in 7% (29/417), postoperative intra-abdominal abscess occurred in 9% (36/417), and postoperative enteric fistula occurred in 1% (3/417). Other septic complications occurred in 22% (93/417) of the operations. At least one postoperative complication occurred in 34% (141/417) of the operations, and re-intervention within 30 days was needed in 16% (65/417) of the cases. Five patients died, four of them due to sequelae of anastomotic leaks. The median postoperative stay was 7 days (2-157).

Table 1: Background data for 32 patients treated preoperatively with anti-TNF-α agents and 385 patients without anti-TNF-α treatment.

	Anti-TNF-α n = 32	No anti-TNF-α n = 385
Age (mean and range in years)	33 (18-62)	37 (8-90)
Gender (female)	34.4% (11/32)	59.2% (228/385)
Duration of disease (mean and range in months)	84 (4-337)	50 (0-444)
Previous CD laparotomy/ laparoscopy	40.6% (13/32)	42.9% (165/385)
Operation urgency		
Acute	25% (8/32)	23.1% (89/385)
Emergent or elective	75 % (24/32)	76.9% (296/385)
Steroids preoperatively	34.4 % (11/32)	34.5 % (135/385)
Steroids, 20 mg or more prednisolone preoperatively	15.6% (5/32)	15.8% (61/385)
Preoperative immunomodulation	87.5% (28/32)	35.8% (135/385)
Preoperative fistula or abscess	34.4% (11/32)	19.2% (74/32)

The values are the medians (ranges) or percentages (numbers).

No differences in the rates of IASCs, septic complications, or any other complications were found between patients with and those without preoperative anti-TNF-α treatment (**Table 2**). Similarly, no differences in complication rates were found between patients on immunomodulation and those who were treated with a combination of preoperative anti-TNF-α and immunomodulation. Treatment with a prednisolone dose of at least 20 mg or more increased IASCs from 11% to 19%

(p=0.04) but was associated with no difference in the rate of septic complications or any complications. In multivariate analyses, treatment with prednisolone 20 mg or more, operation time and colocolic anastomoses were independent predictors of IASCs. Perioperative bleeding was an independent predictor of postoperative septic complications; age, intraoperative bleeding, and duration of operation were predictors for any complication.

Table 2 Potential risk factors for postoperative intra-abdominal septic complications (IASCs) in 417 abdominal operations for Crohn's disease with anastomosis and/or strictureplasty.

		Operations n (%)	Complics. n (%)	Univ. *p*	Odds ratio (95% CI)	Log reg *p*
Sex	Female	249 (59.7%)	34 (13.6%)	0.373	1.379 (0.703-2.704)	0.35
	Male	168 (40.3%)	19 (10.7%)			
Age	< 37 years	208 (49.9%)	36 (17.3%)	0.003	1.020 (0.999-1.042)	0.063
	≥ 37 years	209 (50.1%)	16 (7.7%)			
Dis. duration						
< 55 months		209 (50.1%)	33 (15.8%)	0.040	0.604 (0.291-1.256)	0.177
≥ 55 months		208 (49.9%)	19 (9.1%)			
Previous CD resections						
No		239 (%)	25 (10.5%)	0.150	0.828 (0.407-1.684)	0.602
Yes		178 (%)	27 (15.2%)			
Priority of surgery						
Elective/emergent		320 (76.7%)	40 (12.5%)	0.97	0.834 (0.390-1.784)	0.640
Acute		97 (23.3%)	12 (12.4%)			
Preoperative fistula/abscess						
No		332 (79.6%)	12 (14.1%)	0.606	0.929 (0.431-1.999)	0.850
Yes		85 (20.4%)	40 (12%)			
Steroids > 20 mg						
No		351 (84.2%)	39 (11.1%)	0.040	0.355 (0.167-0.756)	0.007
Yes		66 (15.8%)	13 (19.7%)			
Immunomoduln.						
No		251 (60.2%)	35 (13.9%)	0.263	1.230 (0.613-2.469)	0.560
Yes		166 (39.8%)	17 (10.2%)			
Anti-TNF-α treatment						
No		385 (92.3%)	49 (12.7%)	0.581	1.243(0.314-4.928)	0.757
Yes		32 (7.7%)	3 (9.4%)			

Table 2 is continued on the next page

Table 2 continued	Operations n (%)	Complics. n (%)	Univ. *p*	Odds ratio (95% CI)	Log. reg *p*
Immunomoduln. & anti-TNF-αal					
Neither	251 (92.3%)	50 (13%)	0.268	2.044 (0.465-8.993)	0.344
Both	32 (7.7%)	2 (6.3%)			
Colocolic anastomosis					
No	402 (96.4%)	47 (11.7%)	0.013	0.197 (0.058-0.667)	0.009
Yes	15 (3.6%)	5 (33.3%)			
No. of anastomoses or strictureplasties					
1	384 (92.1%)	45 (11.7%)	0.113	0.456 (0.159-1.305)	0.143
> 1	33 (7.9%)	7 (21.2%)			
Anastomosis technique					
Hand-sewn	254 (60.9%)	27 (10.6%)	0.156	1.130 (0.395-3.234)	0.820
Stapled	140 (33.6%)	22 (15.7%)			
Missing	23				
Duration of operation	200 (47.96%)	33 (16.4%)	0.018	1.010 (1.005-1.015)	< 0.01
< 118 min	217 (52.04%)	19 (8.8%)			
≥ 118 min					
Periop. bleeding					
< 150 ml	212 (50.8%)	23 (10.8%)	0.0291	0.991 (0.499-1.966)	0.979
≥ 150 ml	205 (49.2%)	29 (14.1%)			

Complics.: complications; immunomoduln.: immunomodulators; preop: preoperative; periop.: peri-operative, univ.: univariate analyses; log. reg.: logistic regression analyses.

4.1.2 LIMITATIONS OF STUDY 1

The design of study 1 was retrospective, with all the inherent limitations of this type of design. Follow-up was limited to 30 postoperative days. Selection bias (patients who receive anti-TNF-α are generally those with severe disease), differences in reporting outcomes due to lack of a predefined protocol at the time of the event, and lack of registration of other possible confounding factors, such as disease severity, nutritional status and the anti-TNF-α treatment details, are among the most important limitations of this and other retrospective studies that have investigated this issue. The combination of anastomoses and strictureplasty might have diluted the rate of anastomotic leaks.

4.2.1 RESULTS OF STUDY 2

In total, 14 studies (**figure 5**) fulfilled the inclusion criteria[80,81,90–93,82–89]; of these studies, two studies had only been published as abstracts[86,92] at the time of writing. One of these abstracts was recently published as an original research article[94]. The author of the other abstract [86] was contacted to ascertain the fate of the study, but no

answer was received. The studies included 697 patients in the intervention (anti-TNF-α agents) group and 2345 patients in the control group. Eleven studies included patients with CD, and three included patients with CD, UC, or indeterminate colitis[82,89,91.] These three mixed-population studies included 776 patients, of whom 183 received anti-TNF-α treatment. The patients in the intervention group received their last dose of the anti-TNF-α agent 4 to 12 weeks prior to surgery.

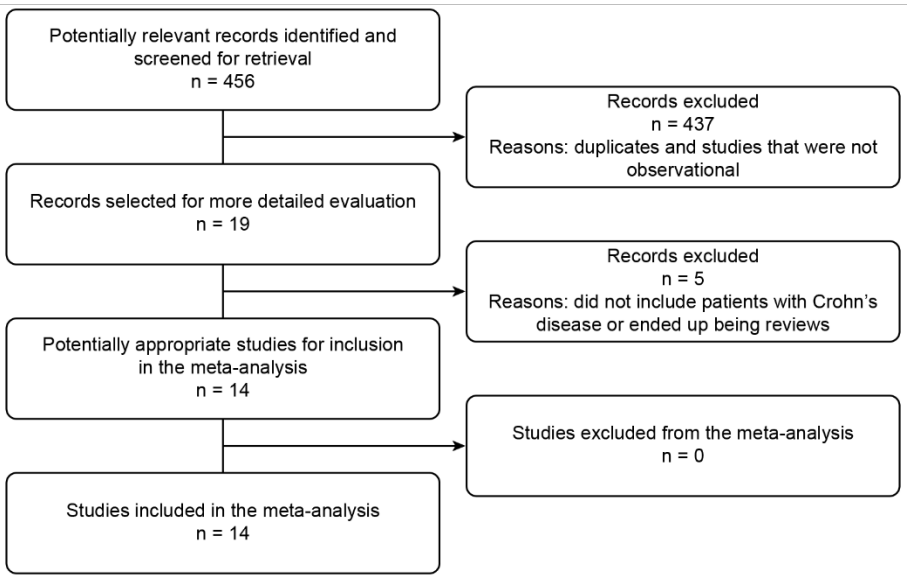

Figure 5: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram implemented in study 2. The diagram shows the results of the search and the reasons for the selection of the 14 studies that were included in the meta-analysis.

All studies were retrospective (**figure 5**). The selection of patients was classified as adequate in all studies. Bias in the outcome assessment was detected in two studies. Three studies[85,86,87] did not adjust for potential confounding factors in an adequate manner. Based on the quality assessment, three studies were classified as having a high risk of bias (≤ 6 stars), seven studies were classified as having a medium risk of bias (7 stars), and four were classified as having a low risk of bias (8 stars).

Table 3: Characteristics of the studies included in the meta-analysis (study 2). Twelve original studies and two abstracts were included in the meta-analysis. One of the included abstracts was published as an original study (Kotz et al.); the other abstract (Brzezinski et al.) was not published. The Newcastle-Ottawa Quality Assessment Scale for Cohort Studies is a tool that facilitates quality control in observational studies. A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories, and a maximum of two stars can be given for comparability.

Author	Quality assessment (Newcastle-Ottawa Scale)	Type of anti-TNF-α agent	Number of patients treated with anti-TNF-α agents with/without other medications	Number of untreated patients (control)
Brzezinski et al. [86] 2002	Selection: *** Comparison: * Outcome: **	IFX	Total: 22 (CD n = 16) +ST: NR +IM: NR	Total: 41 +ST: NR +IM: NR
Tay et al. [90] 2003	Selection: *** Comparison: * Outcome: ***	IFX	Total: 22 +ST: NR +IM: 20	Total: 78 +ST: 14 +IM: 70 No therapy: 7
Colombel et al. [85] 2004	Selection:*** Comparison: * Outcome: **	IFX	Total: 52 +ST: 19 +IM: 19 +ST & IM: 53	Total: 218 +ST: 34 +IM: 8 +ST & IM: 6 No therapy: 33
Marchal et al. [83] 2004	Selection: *** Comparison: * Outcome: **	IFX	Total: 40 +ST: 12 +IM: 24 +ST & IM: 29 Other: 22	Total: 39 +ST: 12 +IM: 11 +ST & IM: 16 Other: 32
Appau et al. [81] 2008	Selection: *** Comparison: ** Outcome: ***	IFX	Total: 60 +ST: 39 +IM: 37 Other: 36	Total: 329 (69) +ST: 253 +IM: 55 Other: 196
Kunitake et al. [82] 2008	Selection: *** Comparison: ** Outcome: ***	IFX	Total: 101 (CD n = 57) +ST: 76 +IM: 37	Total: 312 (CD n = 131) +ST: 240 +IM: 81
Indar et al. [87] 2009	Selection: *** Comparison: * Outcome: *	IFX	Total: 17 +ST: 7 +IM: 5 +St & +IM: 3	Total: 95 +ST: 21 +IM: 15 +St & +IM: 16 No treatment: 43
Regadas et al. [89] 2010	Selection: *** Comparison:* Outcome: ***	IFX	Total: 28 (CD n = 7) +ST: 14 +IM: 11 +ST & IM: 4	Total: 221 +ST: 72 (CD n = 5) +ST & IM: 35 (CD n = 2) No treatment: 114 (CD n = 7)
Nasir et al. [84] 2010	Selection: *** Comparison: ** Outcome: ***	IFX, ADA, CZP	Total: 119 +ST: 37 +IM: 32	Total: 251 +ST: 114 +IM: 83
Kasparek et al. [88] 2011	Selection: *** Comparison: * Outcome: ***	IFX	Total: 48 +ST: 45 +IM: 35	Total: 48 +ST: 45 +IM: 35

Table 3 is continued on the next page

Table 3 continued

Author	Quality assessment (Newcastle-Ottawa Scale)	Type of anti-TNF-α agent	Number of patients treated with anti-TNF-α agents with/without other medications	Number of untreated patients (control)
Canedo et al. [93] 2011	Selection: *** Comparison: ** Outcome: ***	IFX	Total: 65 ST and/or IM: NR	Total: 160 ST and/or IM: 85 Other: 75
Rizzo et al. [91] 2011	Selection: *** Comparison: * Outcome: ***	IFX, ADA CZP	Total: 54 (CD n = 37) +ST: 19 (CD n = 10) +IM:21 (CD n = 18) +ST & IM: 7 (CD n = 5) Other: 11	Total: 60 (CD n = 38) +ST: 29 (CD n = ?) +IM: 6 (CD n = ?) Other: 14
Kotz et al. [92] 2011	Selection: *** Comparison: * Outcome:***	IFX and ADA	Total: 19 +ST: NR +IM: NR	Total: 57 +ST: NR +IM: NR
El-Hussuna et al. [80] 2012	Selection: *** Comparison: * Outcome: ***	IFX and CZP	Total: 32 +ST: 21 +IM: 28	Total: 385 +ST: 75 +IM: 79 +ST&IM: 58 No treatment: 171

CD: Crohn's disease; NR: not reported in the study; +ST: treated with steroids; +IM: treated with immunomodulators. Only patients who received preoperative anti-TNF-α agents are reported; patients who received anti-TNF-α agents after surgery are not reported, as in, for instance, the abstract published by Brezezinski et al.

Effect of anti-TNF agents on anastomotic complications

Eleven studies[80,81,93,82–84,88–92] reported anastomotic complications, which were diagnosed in 45 of 593 patients (7.6%) in the anti-TNF-α group and 143 of 1747 controls (8.2%). Random-effects meta-analyses found no difference between the anti-TNF-α and control groups (RR, 0.91; 95% CI, 0.56-1.47; **figure 6).** There was moderate heterogeneity (I^2 = 44.5%). The results were stable in sensitivity analyses using the OR scale (OR, 0.90; 95% CI, 0.53-1.53), the fixed effects meta-analysis (RR, 0.97; 95% CI, 0.70-1.33), and after exclusion of the trials with mixed patient populations (RR, 1.06; 95% CI, 0.41-2.74). Regression analyses showed evidence of small-study effects (Egger test p = 0.027 (that is, smaller studies might show different, often larger, treatment effects than larger ones). Subgroup analyses found a significant difference between groups of studies stratified by the risk of bias (test for subgroup differences p = 0.004). The studies with a low risk of bias found that anti-TNF-α agents increased the risk of anastomotic complications (RR, 1.63; 95% CI, 1.03-2.60; number needed to harm, 37 patients), but this association was not found in the studies with a medium bias risk (RR, 0.17; 95% CI, 0.05-0.60). **Figure 6** illustrates these findings. It was not possible to extract data on anastomotic complications from the studies with a high risk of bias.

Secondary outcomes

Thirteen studies[80,81,91,82–89] reported the total number of patients with complications (Tay et al. reported only IASCs[90]). The fixed-effects meta-analysis revealed that treatment with anti-TNF-α agents increased the total number of complications (238 of 419 patients (56.8%) versus 589 of 1627 patients (36.2%); RR, 1.25; 95% CI, 1.10-1.43; $I^2 = 81.8\%$), but no such effect was observed in the random-effects meta-analysis (RR, 1.18; 95% CI, 0.86-1.62). There were no small-study effects (Egger test p = 0.459). Subgroup analysis showed a difference between studies stratified by the risk of bias (test for subgroup differences $p < 0.001$). The studies with a high risk of bias found no difference between the anti-TNF-α and control groups (RR, 0.79; 95% CI, 0.58-1.08). Treatment with anti-TNF-α agents increased the total number of patients with complications in studies with a medium or low risk of bias (RR, 1.28; 95% CI, 1.04-1.56 and RR, 1.77; 95% CI, 1.46-2.15). Mortality was reported in 12 studies, and no events were reported in seven studies. In the remaining five studies (all classified as having a low risk of bias), 10 of 231 patients in the anti-TNF-α group (4.3%) and 10 of 792 patients in the control group (1.3%) died. The intervention increased the mortality rate in the fixed-effects meta-analyses (RR, 7.01; 95% CI, 2.75-17.84) but not in the random-effects meta-analysis (RR, 4.80; 95% CI, 0.66-34.82). There was substantial heterogeneity ($I^2 = 53.4\%$) and no evidence of small-study effects (Egger test p = 0.627).

Nine studies[80–84,88,89,91,93] reported other (non-anastomotic) surgical complications, including wound infections, prolonged ileus, adhesions, bleeding gastric ulcers, and wound dehiscence. In total, 74 of 473 patients in the anti-TNF-α group (15.6%) and 180 of 1625 controls (1.1%) experienced other surgical complications (RR, 1.40; 95% CI, 1.05-1.85; $I^2 = 0\%$; Egger test p = 0.545). Major medical complications, including acute renal failure and thromboembolic and cardiovascular complications, were registered in 24 of 458 (4.4%) patients in the anti-TNF-α group and 51 of 1594 patients (3.2%) in the control group. Anti-TNF-α treatment increased the risk of major medical complications (RR, 1.97; 95% CI, 1.23-3.14). There was no heterogeneity and no small-study effect ($I^2 = 0\%$ and Egger test p = 0.083). Forty-nine of 369 patients in the anti-TNF-α group (13.3%) and 41 of 1156 controls (3.5%) developed minor medical complications (RR, 2.37; 95% CI, 1.24-4.50; $I^2 = 37.4\%$; Egger test p = 0.262). The risk of infectious complications did not differ between the anti-TNF-α and control groups (93 of 359 patients (25.9%) versus 261 of 1290 patients (20.2%); RR, 1.15; 95% CI, 0.86-1.53; $I^2 = 33.8\%$; Egger test p = 0.834). Reoperation was reported in five studies and occurred in 21 of 212 patients (9.9%) in the anti-TNF-α group versus 89 of 1106 patients (8.05%) in the control group (RR, 1.09; 95% CI, 0.61-1.95; $I^2 = 19.6\%$; Egger test p = 0.577).

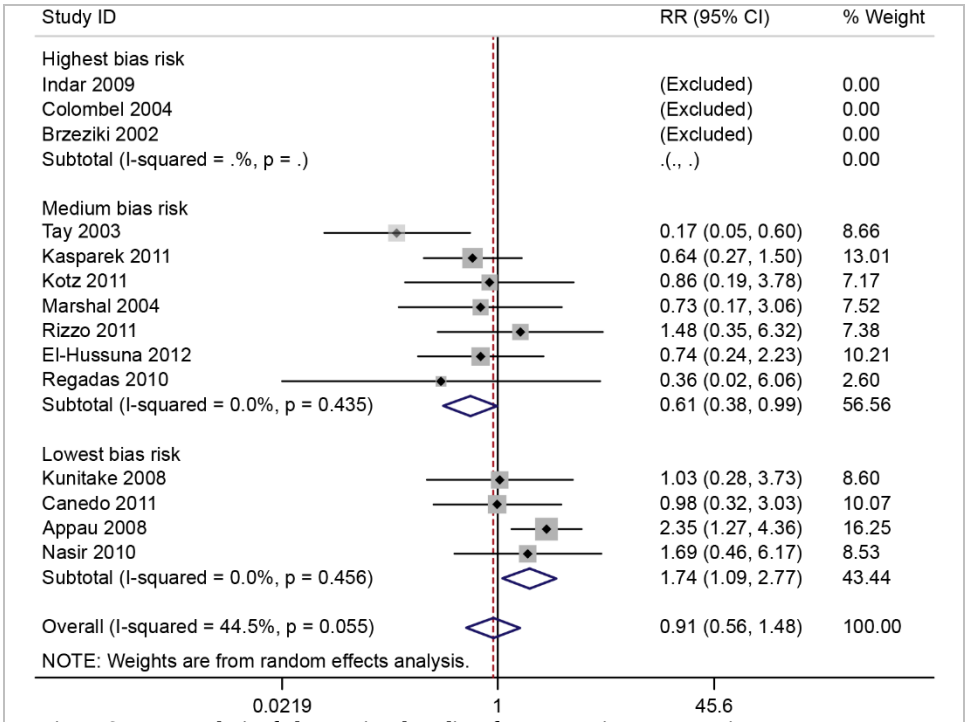

Figure 6: Meta-analysis of observational studies of postoperative anastomotic complications in patients with Crohn's disease. The studies are stratified according to the risk of bias, as assessed using the Newcastle-Ottawa Scale. In the studies with the lowest risk of bias, anti-TNF-α agents increased the risk of anastomotic complications (RR, 1.63; 95% CI, 1.03-2.60), but this increase was not detected in the studies with a medium risk of bias (RR, 0.17; 95% CI, 0.05-0.60). RR = risk ratio.

4.2.2 LIMITATIONS OF STUDY 2

The main limitation of this review is related to the design of the included studies. The observational design entails a risk of bias that cannot be eliminated through adjusted analyses. Moreover, many potential confounding factors were not addressed in all the included studies.

4.3.1 RESULTS OF STUDY 3

As of 4 July 2013, there were 18 retrospective studies on the effect of anti-TNF-α treatment on postoperative complications in CD patients undergoing abdominal surgery[80,81,92,93,95–98,82,83,85–88,90,91]. These studies have been the subject of two systematic reviews[99,100] and six meta-analyses[79,101–105]. The PRISMA diagram is shown in **figure 7.**

The first narrative review by Subramanian et al. was limited to three studies that included 425 CD patients, of whom 108 were preoperatively treated. The authors concluded that the available evidence does not suggest an increased rate of

postoperative complications after preoperative treatment. Ali et al.[100] reviewed eight studies: three on CD patients, four on UC patients, and one with a mixed IBD population. In total, these studies included 1,372 CD patients, of whom 199 were treated with anti-TNF-α. The authors claimed that it was not possible to reach definite conclusions because "the studies are limited by small numbers of patients, disparate comparison groups, different definitions of measured outcomes and varying timeframes of drug exposure and follow-up".

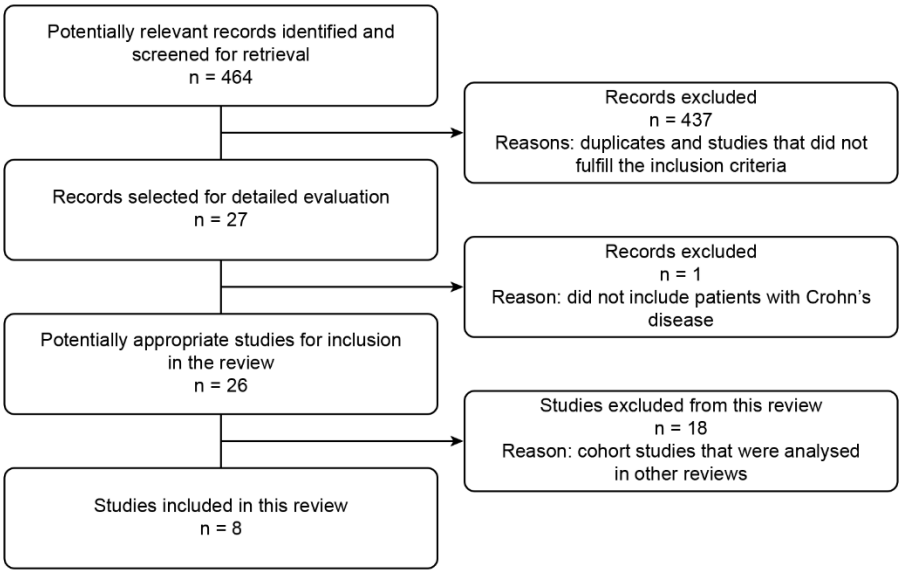

Figure 7: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram implemented in study 3. The figure shows the process used to select the eight studies included in the review.

The major findings of the six meta-analyses are presented in **Table 4**. The first analysis, which was conducted by Efthasami et al.[103], focused on colectomy rates and postoperative complications in UC and CD patients. The method used for quality assessment in this study was basically designed for randomized controlled clinical trials. No sensitivity analysis was conducted to identify studies with a high risk of bias. This study had many crucial drawbacks, including the mixing of retrospective and randomized studies, the mixing of studies on the recurrence of disease with those that examined postoperative complications, and the reporting of results in a non-systematic manner. The study results were difficult to interpret. This study revealed an increased risk for overt anastomotic leaks, pouch-related complications, infections and thrombotic events after anti-TNF-α treatment. Not all relevant studies were included. No subgroup analysis of CD patients was performed, and the results are not applicable to these patients.

Table 4: Meta-analyses of the relationship between anti-TNF-α treatment and postoperative complications in Crohn's disease. A statistical comparison could not be performed due to the use of different inclusion criteria and wide variations in the reporting of the outcome measures.

Study	Disease studied	No. of studs.	Medication	Treated/ Total No. of CD patients	Method of quality assessment	Results after application of quality assessment. [a]
Ehteshami-Afshar et al. [103] 2011	CD & UC	3 CD, 8 UC & 1 mixed	IFX	253/1,151	Jadad	All: OR = 2.11 (CI: 1.02-4.36) Anastomotic: OR = 1.71 (CI: 1.02-2.87) Infectious: OR = 1.56 (CI: 0.71-3.44)[a]
Kopylov et al. [101] 2012	CD	8	Anti-TNF-α agents	423/1,641	NOS (excluded studies were not mentioned)	All: OR = 2.2 (CI: 0.96-5.04) Anastomotic: OR = 1.18 (CI: 0.61-2.30) Infectious: OR = 1.62 (CI: 0.92-2.86)
Rosenfeld et al. [104] 2013	CD	6	IFX	257/1,159	-	Major: OR = 1.59 (CI: 0.89-2.86) Minor: OR = 1.8 (CI: 0.87-3.71)
Billioud et al. [102] 2013	CD, UC & IC	9 CD, 9 UC & 3 mixed	Anti-TNF-α agents	977/4,251 (pure CD patients 549/1,907)	-	All: OR = 1.31 (CI: 0.96-1.77) Anastomotic: *Not stated* Infectious: OR = 1.45 (CI: 1.03-2.05)
Narula et al. [105] 2013	CD & UC	7 CD, 8 UC & 3 mixed		1,146/4,659	NOS	All: OR = 2.19 (CI: 1.69-2.84) Anastomotic: *Not stated* Infectious: OR = 1.93 (CI: 1.28-2.89)[b]
El-Hussuna et al. [79] 2013	CD	11 CD & 3 mixed	Anti-TNF-α agents	679/3,042	NOS	All: RR = 1.77 (CI: 1.46-2.15) Anastomotic: RR = 1.63 (CI: 1.03-2.60) Infectious: RR = 1.15 (CI: 0.86-1.53)

CD = Crohn's disease; IC = indeterminate colitis; NOS = Newcastle-Ottawa Scale; UC = ulcerative colitis; No.: number.

a. The results are expressed as overall complications (All), anastomosis-related complications (Anastomotic) and infectious complications (Infectious).
b. Complications are presented for both CD and UC.

The two meta-analyses from 2012 and 2013 included more than 1,000 patients each. Kopylov et al.[101] addressed many of the limitations of the previous reviews in their meta-analysis. However, these authors excluded abstracts and three relevant studies of mixed populations of patients with CD, UC and indeterminate colitis and did not report the results obtained after the application of quality assessment. Nevertheless,

these researchers reported an increase in infectious complications in addition to trends of increased overall and non-infectious complications. No increase in the risk of anastomotic complications was detected in a subgroup analysis, and the authors noted that the statistical analysis was influenced by discrepancies in the classification of complications.

Rosenfeld et al.[104] found no difference in the rates of major complications, minor complications, re-operation or 30-day mortality between the IFX and control groups. Sensitivity analyses performed by excluding individual studies had no influence on the results. The review excluded some studies that reported the use of anti-TNF-α agents other than IFX. Moreover, it also included abstracts in the results section but excluded them from the analysis, which might be confusing. No quality assessment was reported, and the results obtained after the complications were grouped into major and minor complications were not comparable with those of other meta-analyses. In addition to the above-mentioned drawbacks, the authors admit that the small number of included studies indicates a low power to detect bias.

Three recent meta-analyses included more than 3,000 operations. Billioud et al.[102] examined several studies of CD and UC patients but did not apply any quality assessment or sensitivity analysis. These researchers found an increased risk of infectious complications in CD patients. There was no subgroup analysis of anastomotic complications. Narula et al.[105] performed a meticulous sensitivity analysis and applied a quality assessment by excluding studies with a high risk of bias. These researchers found that treatment with anti-TNF-α agents was associated with increased risks of overall and infectious complications and revealed a trend towards an increase in non-infectious complications. These authors did not analyse anastomosis-related complications, although they included 7 studies that reported anastomotic leak in CD[81–84,88,91,93]. The most recent meta-analysis by El-Hussuna et al.[79] applied quality assessment and sensitivity analysis, excluding studies with mixed populations. After excluding studies with a high risk of bias, the authors found increases in the risks of overall postoperative complications and anastomosis-related complications in patients treated with anti-TNF-α.

4.3.2 LIMITATIONS

Although this study was cited and used as a reference study in an ESCP-ECCO 2016 statement on anti-TNF-α and postoperative outcome[106], it has some limitations, one of which is that it is a narrative review. The included meta-analyses used different outcome definitions and different inclusion criteria and implemented bias control to varying degrees.

4.4.1 RESULTS OF STUDY 4

This pilot study succeeded in recruiting 46 patients, of whom 18 had been treated with an anti-TNF-α agent within 3 months prior to surgery (**Table 5**).

Table 5: Preoperative and intraoperative characteristics of 46 IBD patients treated with anti-TNF-α compared with those of anti-TNF-α-naïve patients.			
Patient characteristics	**Anti-TNF-α treated 18/46 (39.1%)**	**Anti-TNF-α naïve 28/46 (60.9%)**	**Uni-variate**
Age in years (mean ± SD)	38.7 ± 16.36	44.39 ± 12.62	*ns*
Female	11/18 (61.1%)	14/28 (50%)	*ns*
Body mass index (kg/m^2)	24.93 (± SD 6.12)	24.7 (± SD 5.37)	*ns*
Type of disease n (%): Crohn's disease Ulcerative colitis	13/18 (72.2%) 5/18 (27.8%)	19/28 (67.9%) 9/28 (32.1%)	*ns*
Smoking n (%): Non-smoker or ex-smoker Smoker	14/18 (77.8%) 4/18 (22.2%)	22/28 (78.6%) 6/28 (21.4%)	*ns*
Steroids n (%): Immunomodulators n (%):	7/18 (38.9%) 8/18 (44.4%)	9/28 (32.1%) 7/28 (25%)	*ns*
NSAID intake preoperative n (%):	0	1/28 (3.6%)	*ns*
Anti-coagulant intake n (%):	1/18 (5.6%)	0	*ns*
Harvey-Bradshaw Index in CD patients above the calculated median (7.5) n (%):	4/18 (36.4%)	8/28 (61.5%)	*ns*
Preoperative albumin mmol/l (mean ± SD)	33.22 ± 7.53	36.89 ± 4.36	*ns*
Preoperative haemoglobin mmol/l (mean ± SD)	7.86 ± 0.94	8.09 ± 1.01	*ns*
Nutritional risk screening score n (%): No risk Mild Moderate Severe	6/18 (33.3%) 6/18 (33.3%) 3/18 (16.75) 3/18 (16.7%)	20/28 (71.4%) 4/28 (14.3%) 2/28 (7.1%) 2/28 (7.1%)	*ns*
Preoperative parenteral nutrition n (%):	5/18 (27.8%)	1/28 (3.6%)	p = 0.028
Steroid stress dose n (%):	5/18 (27.8%)	0	p = 0.003
Dexamethasone n (%): 4 mg 8 mg	1/18 (5.6%) 2/18 (11.1)	1/28 (3.6%) 9/28 (32.1%)	*ns*
Preoperative epidural analgesia n (%):	5/18 (27.8%)	7/28 (25%)	*ns*
Access to abdomen n (%): Laparoscopic Converted Open	13/18 (72.2%) 3/18 (16.7%) 2/18 (11.1%)	17/28 (60.7%) 2/28 (7.1%) 9/28 (32.15%)	*ns*
Type or resection n (%): SM & IC Colectomy and/or rectal stoma closure	9/18 (50%) 8/18 (44.4%) 1/18 (5.5%)	11/28 (39.3%) 12/28 (42.9%) 5/28 (17.8%)	*ns*
Postoperative epidural analgesia n (%):	6/18 (33.3%)	12/28 (42.9%)	*ns*
Postoperative NSAID used n (%):	0	2/28 (7.1%)	*ns*
Postoperative parenteral nutritional support n (%):	5/18 (27.8%)	1/28 (3.6%)	p = 0.028

All operations were performed with a specialist surgeon in charge. Anti-TNF-α: anti-tumour necrosis factor drugs; ns: non-significant; SM: small bowel; IC: ileocolic.

Background characteristics

The median age was 42.5 years (IQR 23 years), and 25 of 46 patients were female (54.3%). Four of the 18 (22.2%) patients in the anti-TNF-α-treated group had one or more comorbidities compared with 7 of 28 (31.8%) patients in the anti-TNF-α-naïve group. The duration of disease in the anti-TNF-α-treated patients (median 5 years, IQR 7 years) and anti-TNF-α-naive patients (median 8.5 years, IQR 12 years) did not differ. The pre-and intraoperative characteristics of the patients are reported in **Table 5.**

The anti-TNF-α-naïve patients had a higher rate of previous IBD surgeries compared with the anti-TNF-α-treated patients (p = 0.003). The anti-TNF-α-treated patients were more likely to have received preoperative parenteral nutritional support (p = 0.028). Moreover, anti-TNF-α-treated patients with CD had a greater mean ileocaecal/ileocolic resected segment length (mean 31.11 ± 35.51 (SD) cm) versus 27.43 ± 18.83 (SD) cm, respectively; p = 0.036) and were more likely to have a stricturing CD phenotype (10/76.9% versus 8/42.1%, respectively; p = 0.01) compared with the anti-TNF-α-naïve patients. The type of surgical incision and the type of bowel resection were similar in the two groups.

The 18 patients who received anti-TNF-α preoperatively were treated with different types of anti-TNF-α drugs at different doses, and there were wide variations in the interval between the last administered dose and surgery (**Table 6**). Thus, 44% of the anti-TNF-α-treated patients had undetectable drug concentrations in their peripheral blood, and only three of these 18 patients had anti-drug antibodies at the time of surgery.

Surgical stress marker patterns in IBD

Figure 8 demonstrates the surgical stress response according to the levels of the main inflammatory biomarkers. The concentration of TNF-α peaked 6 hours after surgical incision, decreased after 24 hours, and then exhibited a plateau at 48 hours. Similar patterns were observed for the IL-6, IL-8, IL-10, IL-17A, WBC, D-dimer, ferritin and transferrin concentrations and the IL-6/IL-10 ratio. While the CRP concentration peaked 48 hours after surgical incision. The TNF-α/IL-10 and TNF-α/cortisol ratios decreased at 6 hours, began to increase at 24 hours, and reached a plateau at 48 hours. A significant stress response over time ($p<0.01$) was found for all biomarkers except TNF-α, IL-17A and cortisol.

Differences in the surgical stress response between the two groups

The patients treated with anti-TNF-α agents tended to show higher concentrations of most inflammatory biomarkers compared with the anti-TNF-α-naïve patients, as shown in **figure 9**. This difference was more pronounced in the patients with detectable drug concentrations and no anti-drug antibodies. However, the differences were not significant (**figure 9**).

Subgroup analyses

Subgroup analysis was performed by selecting patients who underwent laparoscopic ileocecal/ileocolic resection (12/46) to obtain a homogenous group of patients who had undergone the same type of surgical procedure. Comparing the anti-TNF-α-treated and anti-TNF-α-naïve patients in this subgroup showed no significant difference in the surgical stress response (results not shown).

Postoperative outcome

No difference in the adjusted analyses of the rates of overall complications (27.8% versus 28.6%), superficial SSI (7.8% versus 7.1%), IASC (5.6% versus 7.1%) or readmission (22.2% versus 25%) were found between the two groups. The mean LOS in the anti-TNF-α-treated and anti-TNF-α-naïve groups were 5.33 days (± 2.57) and 6.25 days (± 3.01), respectively, but this difference was not significant.

4.1.2 LIMITATIONS

The main limitations of this study include the small sample size, the heterogeneity of the patient population and the included surgical interventions. The administration of steroids at the induction of anaesthesia and the use of postoperative epidural analgesia in some of the included patients might have affected the results.

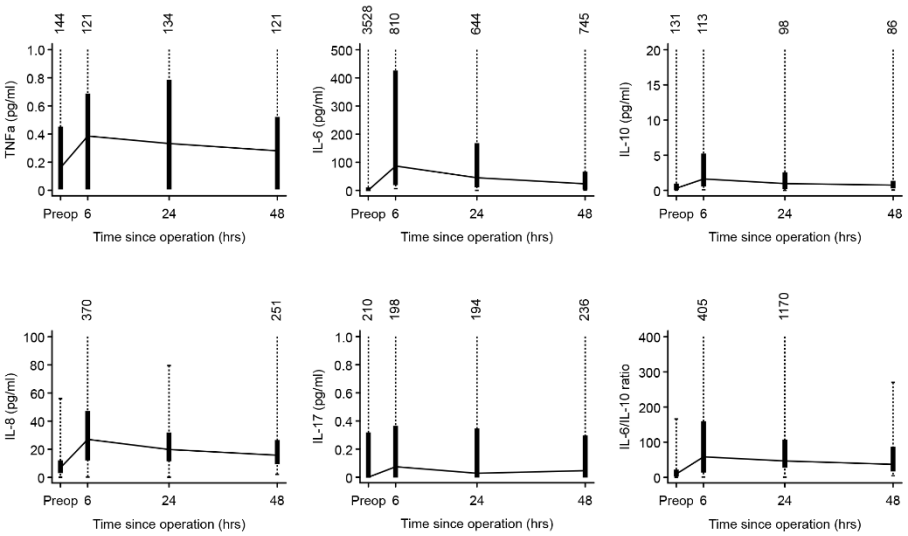

Figure 8: Surgical stress response in 46 patients with inflammatory bowel disease who underwent surgical intervention as part of disease treatment. The main immunological biomarkers of stress are shown. The figure shows that the surgical stress response peaked 6 hours after the surgical incision.
The box shows the medians and inter-quartile ranges, and the numbers above the box show the concentrations of the outliers.

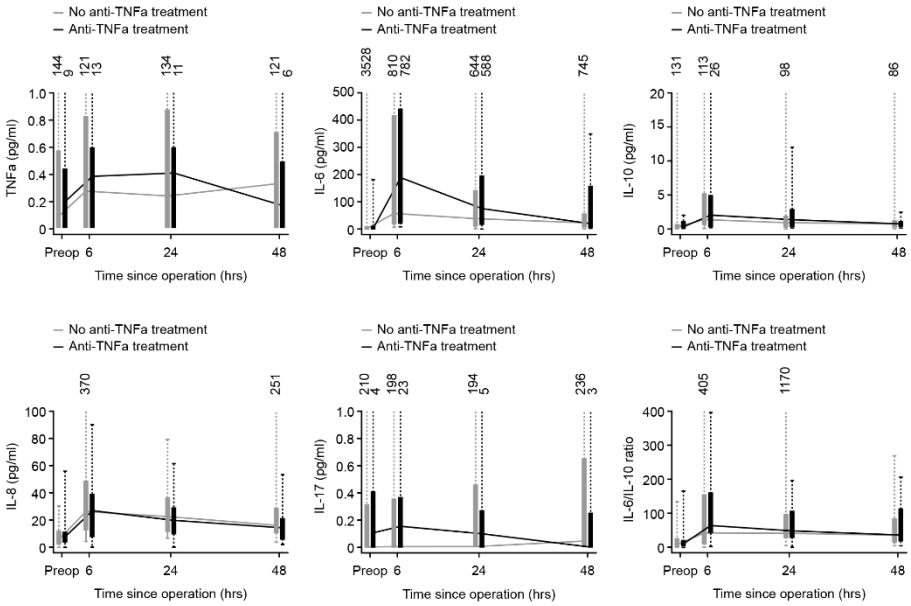

Figure 9: Surgical stress response in patients treated with anti-TNF-α agents and in anti-TNF-α-naïve patients. The figure shows only the main immunological biomarkers of stress (TNF-α, IL-6, IL-8, IL-10, IL-6/IL-10 ratio). The box shows the medians and inter-quartile ranges, and the numbers above the box show the concentrations of the outliers

Table 6: Type of anti-TNF-α agent, duration of treatment, drug concentration and presence of anti-drug antibodies.							
Type	**Dose**	**Last anti-TNF-α dose before surgery (weeks)**	**Drug 0**	**Drug 6 hours**	**Drug 24 hours**	**Drug 48 hours**	**Anti-drug antibodies**
ADA	80	2	0.65	0.65	0.65	0.65	Negative
ADA	40	3	8.49	7.33	7.09	6.13	**Positive**
ADA	40	2	4.25	3.86	3.48	3.73	Negative
ADA	40	1	17.65	16.27	14.42	15.71	Negative
ADA	40	1	2.06	1.41	1.75	0.65	Negative
ADA	40	3	0.65	0.65	0.65	0.65	Negative
ADA	40	1	0.65	0.65	0.65	0.65	Negative
ADA	100	2	2.89	1.34	1.74	1.37	Negative
IFX	300	3	31.00	7.29	15.32	12.95	Negative
IFX	330	2	4.48	4.36	3.56	3.44	Negative
IFX	100	9	0.65	0.65	0.65	0.65	**Positive**
IFX	100	9	0.65	0.65	0.65	0.65	Negative
IFX	350	1	31.00	31.00	21.38	21.94	Negative
IFX	290	8	0.65	0.65	0.65	0.65	Negative
IFX	450	8	0.65	0.65	0.65	0.65	**Positive**
IFX	800	5	17.28	14.17	14.45	15.21	Negative
Golimumab	50	5	1.39	1.06	1.13	0.99	Negative
Combination	700	4	8.05	4.90	5.07	4.58	Negative

Drug 0: drug concentration (μg/ml) before surgery. Drug 6: drug concentration (μg/ml) 6 hours after surgery. Drug 24: drug concentration (μg/ml) 24 hours after surgery. Drug 48: drug concentration (μg/ml) 48 hours after surgery.

A concentration of 0.65 μg/ml refers to undetectable drug concentrations (shadowed). The techniques used for the measurement of anti-drug antibodies are described at the laboratory website (http://www.wieslab.com/diagnostic-ervices/index.php?langId=1&headId=72&subId=143&pageId=195).

5. DISCUSSION

The four studies included in this Ph.D. project show that the effect of anti-TNF-α treatment on the postoperative outcome is due to the interplay of many factors, of which steroid treatment, drug concentration, and the presence/absence of anti-drug antibodies are particularly important.

The relationship of anti-TNF-α therapy to postoperative outcome in patients with CD has been investigated in 30 retrospective cohorts[80,81,90–98,107,82,108–117,83–89], two prospective cohorts[118,119], four experimental studies[120–123], one population-based study[124], one prospective international multi-centre snapshot audit[54], five narrative reviews[99,100,125–127] and eight meta-analyses[79,101–105,128,129] over the last 15 years. In addition to retrospective studies that investigated this issue as one of several possible risk factors for unfavourable postoperative outcome, this group of studies includes studies that focused on UC patients and studies that focused on patients with rheumatoid arthritis. In fact, the effect of anti-TNF-α therapy on postoperative outcome has been the subject of more than 72 scientific papers published in the last 15 years.

Thirteen of the 28 retrospective studies[80,81,90–93,82–89] were discussed and analysed in depth in study 2[79]. The recently published retrospective studies did not show differences in methodology, and the authors of these studies were unable to reach decisive conclusions. However, two of these studies deserve attention. Waterman et al.[96] examined a large series of patients exposed to anti-TNF-α agents (195 patients were matched and compared to 278 controls) and recorded the concentrations of the anti-TNF-α drug and anti-drug antibodies over an extended period of exposure (180 days before and after abdominal surgery). These researchers found that anti-TNF-α agents did not increase the risk of postoperative complications even when the patients were divided into subgroups that included only patients with detectable blood concentrations. The conclusion presented by Lau et al.[107] was completely different. Lau et al. included 150 patients treated with anti-TNF-α agents within a cohort of 217 patients. Half of the anti-TNF-α-treated patients had undetectable drug concentrations in their blood 7 days prior to surgical intervention. For patients with CD, no differences in the postoperative outcomes of the treated and control patients and in the outcomes of the patients with detectable and undetectable blood drug concentrations were found. However, an analysis using a cut-off level of 3 μg/ml for the anti-TNF-α drug concentration revealed that postoperative morbidity (OR = 2.5, p = 0.03) and infectious complications (OR = 3.0, p = 0.03) were significantly higher in the $\geq$ 3 μg/ml group, and higher rates of postoperative morbidity (p = 0.047) and hospital readmissions (p = 0.04) were observed in the $\geq$ 8 μg/mL group compared with the < 3 μg/mL group. The study included only 21% of the patients who underwent surgery during the study period and failed to adjust for many confounders, including the inclusion of patients with undetectable concentrations of anti-TNF-α and untreated patients[130].

Attempts to use prospective nationwide cohorts did not fulfil the hope of reaching decisive conclusions because the two prospective studies reached two diverging conclusions[118,119]. The study conducted by Fumery et al. included 209 patients, of whom 93 were treated with anti-TNF-α within 3 months prior to surgery, and the anti-TNF-α drug concentrations were measured in only 76 of the 93 patients who received anti-TNF-α within 3 months prior to surgery. The authors concluded that treatment with anti-TNF-α was not a risk factor for postoperative complications even in patients who had blood drug concentrations greater than ≥ 3 μg/ml. However, the drug concentration was measured over a wide time window of 3 months after the last anti-TNF-α dose in only a portion of the cohort.
Brouguet et al.[118] found that treatment with anti-TNF-α increases the risk of postoperative complications. The study did not adjust for disease severity, which is a crucial confounding factor in this group of patients because the patients who received anti-TNF-α agents might have been those who did not respond to stepwise treatment beginning with 5-ASA, steroids and immunomodulation and escalating to anti-TNF-α. These patients might have suffered from ongoing inflammation and/or a poor nutritional status (the latter was not assessed using a nutritional index), and neither the drug concentration at the time of surgery nor the level of anti-drug antibodies was measured in the study. Thus, the above-mentioned conclusion is questionable.
Only a few studies reported the rates of proximal faecal diversion by means of protective loop ileostomies[126].

From this perspective, the current literature on CD is complicated by small-sample-size studies with selection bias and heterogeneity in the definition of anti-TNF-α exposure, the outcome timeline, the definition of infectious/non-infectious complications, and the types of surgeries performed. The current literature does not report the combined effect of other potential confounding factors for unfavourable postoperative outcome, such as disease severity/extent, nutritional status, smoking, preoperative drug concentration of anti-TNF-α agents and the presence of anti-drug antibodies[61,126]. Few studies reported the Clavien-Dindo classification of postoperative complications, making it difficult to compare the results. Moreover, anastomotic leak was either not defined or different definitions were used in the studies in which anastomotic leak rates were reported.

The aim of this Ph.D. thesis was to generate evidence that can be implemented in evidence-based medicine (EBM). EBM is the conscientious, explicit and judicious use of the current best evidence in making decisions regarding the care of individual patients[131]. The question of the impact of anti-TNF-α treatment on postoperative outcome was investigated in a retrospective observational study (study 1). However, observational studies might have conflicting results, as shown in studies 2 and 3, in which the published trials were critically appraised. Despite attempts to control bias, many confounding factors that can only be resolved by an RCT or a prospective trial of robust design persist.

Randomization of the patients selected to receive anti-TNF-α therapy followed by surgery in one arm and anti-TNF-α therapy without surgery in the other arm would be the best method for investigating the postoperative outcome in patients with CD. However, this protocol is both impractical and unethical because it would result in some patients undergoing major surgical intervention even though they respond to medical treatment alone, and other patients may require early surgical intervention. Second, the timing of randomization and the choice of when to administer preoperative anti-TNF-α therapy can be difficult. Third, the participation of patients in such studies will be limited by the desire of most patients to avoid surgery. These and other practical difficulties have discouraged researchers from conducting RCTs on this subject.

When an RCT is not feasible, an array of robust techniques can be used in an attempt to replicate the randomization process by creating a control group that is essentially equivalent to the treatment group with respect to known pre-intervention characteristics and assuming that the remaining unknown characteristics will not bias the results. One of these techniques is propensity score matching, which is defined as the probability of assigning a patient to the treatment group conditional on observed covariates (Rosenbaum & Rubin, 1983); this technique controls for pre-intervention differences between the treated and non-treated groups. Although these methods can never ensure the level of validity that could be achieved in an RCT, they are considered robust alternatives when randomization is impractical[132]. This technique was implemented in study 4 to analyse the impact of anti-TNF-α treatment on the concentrations of biomarkers of the surgical stress response. Measurements of the drug levels and antibody status was another feature of study 4.

The maintenance of detectable serum anti-TNF-α trough levels is vital for retaining clinical and endoscopic remission in CD[133]. In the case of a lack of response or a diminishing response to anti-TNF-α agents, the trough level in combination with the anti-TNF-α antibody status might help clinicians make appropriate decisions regarding escalating doses or switching therapies[134]. This was evident in study 4, which showed that patients who received anti-TNF-α treatment within 12 weeks had varying blood drug concentrations and that these varied with respect to the presence of anti-drug antibodies. A repeated measurement of the concentration of the anti-TNF-α drug and anti-drug antibodies prior to the operation and 6, 24 and 48 hours after the time of surgical incision revealed no significant changes in the drug concentration or anti-drug antibody levels despite the observed changes in immunological biomarkers. This result has an important implication for future studies because it shows that a single preoperative measurement of the drug concentration and anti-drug antibody levels at the induction of anaesthesia or within 48 hours of the operation is sufficient for investigating the effect of anti-TNF-α treatment on postoperative outcome.

The dose of the anti-TNF-α agent and the time interval between the last dose and surgical intervention were not reflected in the drug concentration or in the presence or absence of anti-drug antibodies (study 4). Although this finding is not novel, it might nevertheless explain the divergent results of previous studies that reported the

dose of anti-TNF-α and/or the time interval between the last dose and surgical intervention (few studies reported the drug concentration). The withdrawal of anti-TNF-α agents might be guided by measurements of the drug concentrations and anti-drug antibody levels. However, the withdrawal of anti-TNF-α therapy must be weighed against the potential negative effects of gaps in therapy, including immunogenicity and flare of disease. Further research is needed to identify specific groups of patients who may benefit from drug withdrawal.

Although the implementation of the different methodological settings used in the four studies included in this Ph.D. thesis could be considered a limitation, it is also a strength. The studies reflected a maturation of scientific thinking: the analyses and exploration complemented each other, resulting in study 4, which, despite being an exploratory study with a small sample size that involved heterogeneous surgical procedures for CD and UC, contributes to our understanding of the clinical problem and provides a helpful basis for the design of future studies. The lessons learned from studies 1-3 were used in study 4, in which adjustments for all known confounding factors were made based on the extensive literature on the subject. The most important of these factors include disease severity, nutritional status, smoking, the use of concomitant immunosuppressive therapy and the surgeon performing the operation (two surgeons in each centre). The preoperative optimization and the use of anaesthetics, steroids and NSAIDs were documented in details. The protocol was published at clinicaltrials.gov, and complications were defined in the study protocol and classified according to the Clavien-Dindo classification of surgical complications. The implementation of robust statistical methods (study 4) required close cooperation with a statistician, and repeated measurements of the anti-TNF-α, anti-drug antibody and surgical stress biomarker levels required close cooperation with biochemists and specialized laboratories. This design indeed reflects a maturation of the scientific approach and reveals the complexity of the clinical question.

6. CONCLUSIONS AND IMPLICATIONS

The effect of anti-TNF-α therapy on the postoperative outcome in patients with CD reflects the interplay of many factors. The most important of these factors include concomitant steroid therapy, disease severity, nutritional status, smoking, preoperative optimization, the preoperative drug blood concentration levels and the presence of anti-drug antibodies. The preoperative withdrawal of anti-TNF-α is not supported by the current evidence; however, a multi-centre RCT is needed to confirm or disprove this recommendation. To solve the ethical and practical problems associated with RCTs, randomization should take place when patients on anti-TNF-α therapy need surgical intervention.

7. PERSPECTIVES

An RCT in which patients with CD who are receiving anti-TNF-α therapy are randomized to either experience the withdrawal or the continuation of anti-TNF-α therapy prior to elective ileocaecal/ileocolic resection or hemicolectomy is needed. Such an RCT would make it possible to determine the effect of anti-TNF-α therapy on the postoperative outcome. A large sample size, preoperative measurements of the drug concentration and anti-drug antibody levels, and a systematic assessment of the disease severity, nutritional status and preoperative optimization are mandatory for such a study. These considerations will require international multi-centre cooperation over many years because not all CD patients need surgical intervention, a small fraction of those who require surgical intervention receive preoperative anti-TNF-α therapy, and an even smaller fraction of patients undergo ileocaecal/ileocolic resection and hemicolectomy.

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APPENDIX A

The following studies were included in the Ph.D. project:

1) El-Hussuna A, Andersen J, Bisgaard T, Jess P, Henriksen M, Oehlenschlager J, Thorlacius-Ussing O, Olaison G. **Biologic treatment or immunomodulation is not associated with postoperative anastomotic complications in abdominal surgery for Crohn's disease. Scand J Gastroenterol.** 2012 Jun;47:662-8.
2) El-Hussuna, A. Krag A, Olaisson G,Bendtsen F, Gluud, LG. **The effect of anti-tumor necrosis factor agents on the postoperative complications in Crohn's disease patients undergoing abdominal operation: Systemic review and meta-analysis**. Dis Colon Rectum 2013; 56:1423-33
3) El-Hussuna, A. Theede K, Olaisson G. **Increased risk of post-operative complications in patients with Crohn's disease treated with anti-tumour necrosis factor α agents - a systematic review.** DAN MED J 2014;61:A4975
4) El-Hussuna, A., Qvist, N., Zangenberg, M.S, Langkilde, A.,Siersma, V, Hjort, S, Gögenur, I. **No effect of anti-TNF-α agents on the surgical stress response in patients with inflammatory bowel diseases undergoing bowel resections: A prospective multi-center pilot study** (submitted).

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